Monitoring the Adaptation to Experimental Pain Using Functional Near Infrared Spectroscopy

A Thesis

Submitted to the Faculty

Of

Drexel University

By

Daryl Omire-Mayor

In partial fulfillment of the

requirements for the degree

of

Doctor of Philosophy

June 2017

Dedications

To my beloved parents Mejebi & Lucky Mayor for their unconditional love and support,

and to my brothers (Daniel, David, Donald) and sister (DianneMarie) for their love, patience, and

encouragement.

Acknowledgements

I would like to thank my advisor Dr. Kambiz Pourrezaei for his encouragement, guidance and support throughout my 5 and a half years at Drexel. Not only has Dr. Pourrezaei encouraged me within the scope of research, but he has been extremely supportive in helping me to shape my career path appropriately and achieve goals that surpassed what I had in mind. I was honored to have the opportunity to work under him as a part of the Drexel imaging group.

I would also like to take the opportunity to thank Dr. Banu Onaral who graciously offered her guidance and support in motivating me to explore research beyond the Drexel walls, and engage in international collaborations.

I am also thankful to my committee members: Dr. Meltem Izzetoglu, Dr. Ahmet Saçan, Dr. Maria Schultheis and Dr. Fengqing Zhang for graciously giving their time in my years at Drexel University to help me develop from a graduate student, to a PhD candidate, all the way through completion of my dissertation.

I am thankful to the PhD students who graduated before me, Dr. Zeinab Barati and Dr. Ahmad Pourshoghi who continued to advise me after they graduated from Drexel University.

I also extend my thanks and gratitude to the Drexel University School of Biomedical Engineering, Science & Health Systems for their financial support in my time at Drexel, and helping to mold me into a future biomedical scientist.

I would like to thank my lab colleagues that I had throughout my time at Drexel University: Rutvi Vyas, Kanghee Lee, Esther Lim, Pranali Mulay, Ardy Wong, Minakshi Mohanty, Pooja K.J., and Michal Swoboda. All of them continuously put in several hours in hardware development, subject recruitment, experiment conducting, and making my time as a

part of the Drexel Cognitive Neuroengineering & Quantitative Experimental Research group more enjoyable.

Lastly, a very special thank you goes to Derrick Polk, Konrad Thiessen, and all of my friends who supported me emotionally throughout my graduate studies.

Table of Contents

List of Tables

List of Figures

ABSTRACT

Monitoring the Adaptation to Experimental Pain Using Functional Near Infrared Spectroscopy

By: Daryl Omire-Mayor

Background: Despite pain being one of the more thoroughly studied phenomena, it is still one of the more complex and least well understood. Studies of late, in particular imaging studies, have made an attempt to take individuals' subjective response to pain into consideration, while observing how their bodies respond physiologically. This has led to our lab's focus on the adaptation to pain. As adaptation is the reduction of the response to a given stimulus over time, monitoring the adaptation to experimental pain over time using functional near infrared spectroscopy shows the change in autonomic response with each additional exposure. Doing this, while also observing the change in perception to each additional exposure, can further progress the research of pain imaging, and more importantly, show how changes in physiological response and perceptive response correlate over time.

Methods: In the investigations outlined in the aims of this thesis, experimental pain (the cold pressor test in a repeated-measures fashion) was used to conduct experiments in healthy subjects. In conjunction with this, the McGill Pain Questionnaire and State Trait Anxiety Inventory were employed during experimentation as well. In post experimentation analysis, multilevel modeling, linear regression and clustering were the main techniques used to understand how the pain response changes with each additional exposure to a stimulus.

Results: These statistical techniques yielded results that suggest that with each additional exposure to noxious stimulus, the magnitude of a subjects' autonomic response continues to diminish. Through the measures of perception, the subjects did not show significant change with each additional exposure; although, there was a trend demonstrating that the subjects felt the pain with slightly less intensity with each additional exposure.

Conclusions: From interpreting the results of the studies performed as a part of this thesis, it is evident that functional near infrared spectroscopy is a powerful enough imaging tool to monitor adaptation to repeated exposure of stimuli. To further understand how perception and autonomic response relate, more studies with a greater number of subjects will give greater understanding of the connection (or lack thereof) between the two.

Chapter 1

1.1 Background

Pain is a complicated phenomenon in that it is necessary because it gives the body awareness of when it is danger, or any state that deviates from stable and requires correction. Even further, acute pain is adaptive and results in survival actions being taken such as escaping behaviors. In contrast, chronic pain is maladaptive causing suffering and a great reduction in life expectancy (Diatchenko, Nackley, Tchivileva, Shabalina, & Maixner, 2007). The issue with pain in general is that the human pain conditions present in a multitude of biological and psychological phenotypes, in particular, in idiopathic pain disorders. Environmental events that invoke tissue injury and psychological distress also add to the difficulty for diagnosis, due to the way that they can influence amplification of the way that pain is experienced.

Recently, there has been increased interest in the role of both sodium channels and calcium channels as mechanisms behind the development of pain, specifically chronic pain. Voltage-gated sodium channels (VGSC), post nerve injury, have been proven to show changes in plasticity after nerve injury through their expression. Both Nav 1.7 (*SCN9A gene*) and Nav 1.8 (*SCN10A gene*) (sodium ion channels that are expressed at high levels in nociceptive neurons) have been linked with excitability, as well as increased transmission of nociception (pain). Nav 1.7 has even been said to show genetic linkage when it comes to certain pain diseases (Diatchenko et al., 2007). These sodium channels play an important role as damage to them sheds light onto their central activity, as it ultimately can lead to things such as allodynia and hyperalgesia. (Dickenson, 2011; Phillips, Drevets, Rauch, & Lane, 2003)

Calcium channels, in a similar fashion as sodium channels, take part in neuronal excitement (Dickenson, 2011). What calcium channels do is allow for the neurotransmitter release from the

central terminals to the spinal neurons (Dickenson, 2011). This interplay leads to activation of the sensory signals in the CNS. The problem with pain comes in when looking at the major transmitter receptors, such as the N-methyl-D-aspartate (NMDA) receptor for glutamate. It is the constant firing at these receptors that leads to chronic pain, and similar pain related issues when dealing with other diseases. This is the reason why most medications or treatments for pain involve antagonists to receptor sites on the NMDA receptor complex and similar receptors. (Chambers et al., 1999; Ledoux, 2000)

Recall that NMDA receptors are trans-membrane receptors. An ion channel starts off with a magnesium plug. When you get the nociceptive stimulus that comes in, glutamate initially releases the magnesium plug from the central core of the NMDA receptor. Following that, glutamate and glycine and other pro and anti-inflammatory pain mediators can influence the NMDA receptors while glutamate binds intimately with the NMDA receptor. This allows calcium, sodium and sometimes potassium to flow through the necessary channels and ultimately leads to post-synaptic changes that result from pain caused by nerve damage. (Dickenson, 2011)

To corroborate this finding of NMDA, a study was conducted by Nair et. al to look at the elevation of glucocorticoids due to stress and how this increases the proliferation of microglia through NMDA receptor activation. This study used physiological stress to increase the corticosterone level in mice CNS. The stress induced proliferation of microglia as a function of NMDA receptor stimulation; however, blockage of the NMDA receptor prevented stimulation of the microglia, thus demonstrating that the ability of drugs, such as NMDA receptor antagonists, to affect NMDA, can have a significant effect of stress processing and microglia (reticuloendothelial system) stimulation. (Nair & Bonneau, 2006)

When taking a more in depth look at how noxious stimuli is processed, we see that there is a much bigger and more complex picture than only looking at the NMDA receptor. First, we start in the periphery, with an injury. This injury can be the result of direct trauma, vascular damage, direct skin trauma, damage to the neuron itself, the primary afferent neuron injury, etc. This injury leads to a release of neuronal and non-neuronal ligands. These ligands in the primary afferent neuron (PAN) include Substance P, CCK, ATP, glutamate and many others. Some non-neuronal ligands include acetylcholine, amino acids (glutamate), cytokines and opioids, as well as many others. These ligands have a number of effects on receptors on primary afferent neurons and various other afferent neurons. The transducer channels that help to advance this signal include the sodium and calcium channels, as well as acid-sensing ion channels (hydrogen channels) and potassium channels and a few others, all of which begin the initial processing of the nociceptor transmission. The receptors themselves on the PAN that act in the initial response to the trauma are bradykinin, histamine, serotonin, protease, etc. These are all a part of generating the initial inflammatory reaction that occurs peripherally after a pain experience. (Rainville, 2002)

After the initial observation of noxious stimuli, the PAN sets up the nociceptive signal. A-delta and C fibers access higher order neurons by synapse. This occurs in the superficial layers of the dorsal horn gray matter. In this superficial layer, the area of focus is the rexed laminae, in particular, the substantia gelatinosa. A-delta and C fibers synapse high up in rexed laminae, whereas the A-beta fibers synapse deeper. This is first order to the second order of neurons. This synapse in the dorsal horn is an extremely pivotal area in pain signaling upwards and downwards as well. The signal comes towards the end of the PAN and at that synapse, there is a release of pro-nociceptive substances, more importantly substance P and glutamate. At the same time in the pre-synaptic membranes, not only are there pro-pain neurotransmitters and inflammatory mediators,

but also there are anti-pain modulators as well such as GABA, glycine, serotonin, noradrenaline and endogenous opioids which are important in this area. The main central post-synaptic receptor in transmission of pain fibers is the NMDA receptors. (Rainville, 2002)

The post-synaptic changes that occur essentially are the systems that are activated to set up second messengers, which leads to an influx in calcium by way of a number of mechanisms. The IP3 mechanisms, PKC, PKA, etc. All of these mechanisms give rise to the influx of calcium, which ultimately leads to membrane hyperpolariziation and continuation of the neurotransmission of pain. On the postsynaptic side, there are important receptors and channels, gaba, delta and mu opiate receptors, etc. where analgesic efforts can be focused. Gaba A and gaba B receptors, NMDA receptors, opioid receptors will all aid in the effort of finding ways to relieve pain. In addition there are alpha-2 and NK1 receptors, but these are outside of the scope of this paper. (Rainville, 2002)

After the second order neurons, the pain pathways need to get up to the higher centers of the brain, so they need to travel in multiple ascending tracts. The ascending spinal tracts are spinaothalamic, tracts which act as a mediator to the experience of sensations, spinoreticular tracts which responds to noxious stimuli and provide signals to the autonomic centers in the brainstem, spinomesencephalic, which associates itself with the periaqueductal grey matter (PAG) and has neurons that respond to noxious or innocuous stimuli, and spinoparabrachial tracts that have an effect on autonomic changes and emotions in pain, and are primarily involved in nociceptive activity. Once the pain signal reaches the thalamus mid-brain, higher supraspnial projections occur, and from there, the spinothalamic region projects the signal out to the cortical areas and the somatosensory cortices, S1, S2, ACC, PFC, and the insula. Spinoparabrachial tracts project to the

thalamus and amygdala, and the spinomesencephalic region projects to the PAG and reticular activating system, which can add to the excitement resulting from noxious inputs. (Rainville, 2002)

These higher centers help to form what Melzack termed as the pain matrix. It is not simply a group of areas in the brain that are linked together. It should be thought of as something that is fluid and unique to each individual, as we all have different specificities when it comes to our pain matrix (cortical areas: sensory cortices S1 and S2, the anterior cingulate cortex, the prefrontal cortex, and the insula; subcortical areas: thalamus, amygdala, hippocampus, basal ganglia). This is a great topic of interest particularly now with advances in brain imaging as these areas can be monitored over time. (R Melzack, 2001)

The descending pathways of pain include the PAG, which deals with the release of opioids, the nucleus raphe magnus, which releases serotonin, the locus ceruleus which releases noradrenaline, and lastly the rostroventral medial medulla which leads to the last part of the descending pathway, the serotonergic facilitatory pathway that terminates in the spinal cord. It makes sense to say that the activity surrounding these descending pathways increases post-injury as that is when the ascending and descending pathways come into play; however, when things such as fear and anxiety accompany pain, they (at times) amplify this pain due to the link the pathways have between the emotional states and pain level. Because these descending facilitators are independent of long-term potentiation (LTP: an extended time-period where synapses between nerve cells are strengthened) and the initial pain response, they add to the complexity of pain and its intensity. When thinking of both the ascending and descending pathways in conjunction with one another, the gate control theory gives a big picture idea of pain processing. (Dickenson, 2011)

Interpreting the ascending and descending pathways of pain gives a very holistic picture to the bodies response to pain; however, missing from this description of the physiological response

to pain, is the subjective perceived response to pain. This is subject to several environmental factors that are not standardly experienced by every individual, and warrants further investigation when seeking to understand pain in full.

The Cognitive-Emotive Dimensions of Pain Perception

Emotional perception includes three processes that follow the presentation of an emotive stimulus (Goldstein N., 2002; Phillips et al., 2003). First, the stimulus is identified and evaluated for its emotional significance. Subsequently, affective states are generated in response to the stimulus. These affective states involve both autonomic, neuroendocrine, and somatomotor (e.g. facial, gestural, vocal, and behavioral) responses and conscious emotions. These emotions can then influence the initial identification and evaluation of emotional stimuli. A third process follows in which the affective state and emotional behavior are regulated per contextual data and often by means of inhibition and modulation of the first two processes.

Physiological feedback measured from the central and peripheral nervous system was first used to assess the emotional perception of painful stimuli; however, researchers could not discern between the emotive stimulus and the subsequent affective state (Phillips et al., 2003). Instead, animal and human studies have identified neural regions associated with the identification and neurobiological response to emotive stimuli. The regions of the brain that aid in the identification of external stimuli, the production of the following affective states, and the mediation of autonomic responses to external stimuli are: the ventral system, amygdala, insula, ventral striatum, and ventral regions of the anterior cingulate gyrus and prefrontal cortex (Phillips et al., 2003). The amygdala appears to be associated with the emotional perception of facial expression and gaze. Furthermore, evidence indicates the role of the insula – a network including the ventromedial prefrontal cortex, amygdala, hypothalamus and periaqueductal gray – in the orchestration of autonomic responses to

stimuli identified and evaluated as threatening. Studies in humans with focal brain lesions corroborate these findings (Phillips et al., 2003). The dorsal system, including the hippocampus, the dorsal anterior cingulate gyrus, and the prefrontal cortex integrate cognitive processes with emotional information. These brain regions are also associated with executive functions such as selective attention, planning, and conscious regulation of affective states (Phillips et al., 2003).

Given the level of regulation of these affective states, it would appear that great insight into what pain is has been observed; however, the subjectivity of it has not been addressed. The reason it is essential to look at perception on a subjective basis is because individuals can, in a sense, control the way that they perceive things. This leads into perceived controllability, which is the notion that those who believe that they can control an aversive event from taking place, show less autonomic arousal and focused impairment than those who do not believe that they can control the occurrence of said aversive event, although both groups remain vulnerable to the same stimuli. (Bandura & Wood, 1989)

An additional category of pain that can lead to less autonomic arousal is pain adaptation. Adaptation can be defined as a reduction in response to repeated exposure to a given stimuli. In the case of repeated exposure, while the perception of pain may change, the external stimuli itself does not necessarily change. What this means is that the body still must react appropriately to stimuli. Due to this autonomic response based on environmental factors, several brain imaging techniques have emerged as being potentially useful in understanding pain by investigating the correlation of an autonomic response to external factors, and how it relates to perception (Bandura & Wood, 1989; Salomons & Johnstone, 2007). Conducting a study on pain adaptation would yield greater understanding of the reciprocity between pain perception and the physiological response.

1.2 Pain Adaptation and Problem Statement

Adaptation to pain is a defense mechanism in which the body aims to reduce the amount of pain caused to the body as a result of a given stimulus (Hodges & Tucker, 2011). This involves several different bodily reactions that can occur individually or in conjunction with one another: (1) the activity within or between the muscles engaged as a results of the stimulus is redistributed, (2) mechanical behavior is also modified (this is seen as stiffness or change in movement, (3) the previous two resulting in the body protecting itself from further pain/injury, (4) changes from (2) occur at multiple levels of the motor system and are not restricted to one area, (5) in the short term this is greatly beneficial; however, in the long term, it is not ideal to remain in a situation where the body is forced to adapt to pain, given the adjustments that the body makes seen in (1) and (2) (Hodges & Tucker, 2011). Due to the amount of bodily changes that occur because of adaptation, further understanding how the body adapts to pain can give great insight into how one experiences pain. From this motivation stems the focus of this thesis: to **Monitor and evaluate the effects of repeated exposure to experimental pain (both noxious and innocuous stimuli) on the hemodynamic response, using functional near infrared spectroscopy.** Noxious stimuli can be defined as a generally painful stimulus, while innocuous stimuli can be thought of as a stimulus that is generally not painful.

1.3 Objectives

The primary purpose of this PhD research is to monitor the fNIRS signal over time for an objective assessment of adaptation to pain.

Given the flexibility and usability of fNIRS, we plan to take advantage of this and pursue the monitoring of this signal in the assessment of the adaptation to pain. Further, we look at

subjective measures of pain to compare to the signal, to ensure that the objective measure considers the subjective nature of pain. The goal of this research is to assess the usability of fNIRS measurements, as a monitor of adaptation to stimuli over time with fNIRS. This will be done by visualizing the changes to the hemodynamic response over time in the prefrontal cortex regions.

1.4 Specific Aims and Research Hypotheses

SPECIFIC AIM 1. fNIRS and Adaptation. The goal of this specific aim is to measure adaptation to noxious and innocuous (non-painful) stimuli (with the cold pressor test at 4°C and 15°C respectively) through measurements from the fNIRS signal. Within this aim, we first want to identify whether adaptation is observable by the fNIRS system. Looking at features (identified from previous work) of the fNIRS signal across multiple trials after repeated measures testing, we will see if trends of adaptation occur. The hypothesis here is that adaptation will show through a diminished response over time. The second part of this aim would include comparing noxious stimuli to innocuous stimuli (4°C cold pressor test and 15°C cold pressor test). 15°C was used as the innocuous stimuli as this is CPT level is general considered non-painful. We hypothesize that adaptation will not look the same in both cases, but rather that in the case of 4°C, adaptation will be clearer than the adaptation observed in 15°C (if adaptation occurs at all at this temperature). With these two points of focus, we will gain initial insight into how the hemodynamic response changes over time with repeated exposure to stimuli.

SPECIFIC AIM 2. Perception of Pain and Adaptation. In this aim, we want to look specifically at the relationship between the perceived adaptation to pain and the fNIRS signal. This will be done through the comparison of the signal features that were deemed significant (based on channel and chromophore; Oxyhemoglobin: HBO_2 or Deoxyhemoglobin: HB) in AIM

1 and two psychological measures. The first of which is Short-Form McGill Pain Questionnaire, which includes the visual analogue scale (Kabat-Zinn, 1982; R Melzack, 1987, 2001; Ronald Melzack & Wall, 1996). This is a survey created by Robert Melzack that is widely used in pain medicine and research to better understand patients pain experiences. The second is the State Trait Anxiety Inventory (Spielberger R.; Lushere, R., 1971). This inventory, which is measured before and after experimental sessions with repeated exposure to stimuli, will give us a measure of subjects' anxiety before and after pain testing to see if there is a significant change or trend in anxiety, as well as if it correlates to the changes in the fNIRS signal. In particular, the focus will be on state anxiety because that is indicative of present anxiety level, versus trait anxiety which relates more to a generalized anxiety experienced by an individual outside of the present state (Spielberger R.; Lushere, R., 1971). We hypothesize that there will be a correlation between perception and the hemodynamic response seen in the fNIRS signal. The evaluation of the hemodynamic response in this aim will show if the change in the hemodynamic response seen in AIM 1, has a strong relationship with the way that pain is perceived by subjects.

SPECIFIC AIM 3. Exploratory Analysis of fNIRS Clustering in Adaptation. The intention behind this aim is to see if the fNIRS data can be grouped based on trial number of the repeated measure experimentation (i.e. does the data from the first trial look different from the data in the last trial). This will be done using the unsupervised machine learning technique of clustering, and we hypothesize that earlier trials will be easily differentiated from the later trials through clustering. If clustering can differentiate between trials undergone by subjects based on the hemodynamic response data, this will suggest that the fNIRS system is sensitive enough to observe a reduction in response to stimuli (adaptation) over time through monitoring repeated exposure to stimuli.

1.5 Advancements in Pain Research

The public health burden of chronic pain is undeniable. Not only is pain related to a breadth of disease and injury, but chronic pain can also be the disease, itself. Chronic pain is pervasive. According to the Institute of Medicine of The National Academies at least 100 million Americans suffer from some chronic pain syndrome—a number that trumps the sum of patients suffering from heart disease, diabetes, and cancer combined (American Cancer Society, 2014; Institute of Medicine of the National Academies, 2011; Roger et al., 2011; Yang et al., 2013). The prevalence of chronic pain compromises the efficiency of an already overtaxed healthcare system. According to a 2003 nationwide survey, 63 percent of pain sufferers have visited their family physician for their condition, 40 percent sought out further medical care with a specialist, and at least 15 percent visited a physician who specializes in pain management, in particular. (Hart, 2003)

Over the last two decades, many imaging tools have emerged that are gradually providing qualitative and quantitative insight into brain activation in response to noxious stimuli, as well as an understanding of the underlying cause of chronic pain. Both positron emission tomography (PET) and blood oxygen level-dependent functional MRI (BOLD-fMRI) have played major roles in increasing our knowledge of neuronal and vascular processes that are taking place in the brain and their correlation with the sensation and perception of pain. In particular the results of these studies have pointed to the existence of a pain matrix (i.e. bilateral thalamus, insula, anterior cingulate, SII somatosensory cortex and other supra-spinal structures) (Denk, McMahon, & Tracey, 2014). The goal of many contemporary pain treatments is to influence and interact with the operation of this matrix. Most importantly, it is hypothesized that the malfunctioning of this matrix is the main culprit in many chronic pain states such as phantom limb syndrome and fibromyalgia. It has been postulated that appropriate modification and manipulation of this matrix

by chemical, electrical, biofeedback, and/or mindfulness meditation may provide new approaches for treatment of the many presently untreatable chronic pain cases. Activations of these areas are directly correlated to the intensity of an external noxious stimuli as well as pain perception, which result in the encoding of pain intensity felt/perceived by the individual. (Casey, Minoshima, Morrow, & Koeppe, 1996; Disbrow, Buonocore, Antognini, Carstens, & Rowley, 1998; Tracey et al., 2000)

At present the gold standard for quantitative assessment of pain relies on self-reporting questionnaires. Since pain is a very subjective, private and an affective matter, therefore, self-reporting is subjective, mood dependent and not reliable – particularly in cases of children and demented subjects deemed unreliable. Thus, the development of reliable tools for objective assessment of pain has been of great interest. In addition to self-reporting, in a typical pain clinical setting, physiological parameters (such as heart rate, blood pressure, respiratory, galvanic skin response and cutaneous blood flow) and behavioral measures (such as facial grimacing and guarding the painful area) have been utilized for diagnostic purposes. However, based on clinical experiences, these measures are found to be both unreliable and non-specific. (Ranger & Gélinas, 2014)

Initially positron emission tomography (PET) was used to image the activated areas of brain in response to noxious thermal stimuli (Borsook, Sava, & Becerra, 2010). These studies provided some of the earliest evidence for the present day understanding of the brain activation in response to painful stimuli. However, a major problem with PET is its reliance on radioactive contrast agent. The recent and continuous advances in functional magnetic resonance imaging (fMRI) have made this technique the primary brain imaging modality. Using these two modalities, a correlation between activated brain areas and application of noxious stimuli has been established.

Further, a relation between the BOLD signal acquired by fMRI and subjects' self-report has also been identified. (Porro, Cettolo, Francescato, & Baraldi, 1998)

An area that recently has had a major impact in neuroscience in general and in neuroimaging has been the deployment of machine-learning techniques for analyzing neuroimaging data. Marquand et.al. used machine-learning algorithms, namely the Gaussian process (GP) models, multivariate regression and probabilistic classification, to analyze the fMRI data obtained from the whole brain. In their study, they show that GP models can predict subjective pain ratings on the same individual. They favorably compared their results with the state of the art support vector machine (SVM) algorithm. (Marquand et al., 2010)

In another fMRI study whole-brain activity patterns have been used to train a support vector machine (SVM) to distinguish painful from non-painful thermal stimulation (Brown, Chatterjee, Younger, & Mackey, 2011). The generated model in this study is not limited to the individual itself and has been verified on different groups of subjects. They have reported an accuracy of 81% at distinguishing painful from non-painful stimuli.

In yet another fMRI study, machine-learning–based regression technique LASSO-PCR (least absolute shrinkage and selection operator-regularized principal components regression) was able to separate hot from mild thermal stimuli with an accuracy of 94% accuracy (Wager et al., 2013; Wager, Atlas, Leotti, & Rilling, 2011). These studies demonstrate that neuroimaging biomarkers are consistent enough between subjects to create an algorithm that performs with high accuracy when trained on a particular group of subjects, with another separate group serving as the testing group (Brodersen et al., 2012). While both PET and fMRI are very powerful imaging modalities with great depth of penetration and spatial resolution, both modalities are expensive and are not designed for frequent clinical use. Later in this section in the discussion of near infrared

spectroscopy (NIRS) we will see that some of the same similar features can also be measured with NIRS, which can be effectively used in a community clinical setting. (Borsook et al., 2010)

Complementary to the above imaging techniques that focus on blood flow, electroencephalography (EEG) and magnetoencephalography (MEG) provide information regarding real-time neuronal activity. In addition, EEG is relatively inexpensive and has a long history of being used in clinical settings. Still, EEG is very sensitive to electrical noise and motion artifact and typically needs a shielded room. Recently, great improvements have been made in addressing some of the challenges associated with EEG. As for MEG, it is fairly expensive, as it requires a room with magnetic shielding. (Chizh & Hobson, 2007)

Non-invasive optical modalities such as infrared thermography and laser-doppler imaging provide "real time" changes in blood flow, and can assess nociceptor activation and provide pharmacokinetic information (Chizh & Hobson, 2007). A major shortcoming of both techniques is that they can only provide signals emanating from superficial tissue layers. Each of these techniques and their separate focus provide great insight into the pain matrix. Researches utilizing these modalities have increased our basic understanding of the pain matrix and the underlying causes of various chronic pain states.

Another useful technique for neuroimaging of pain is near infrared spectroscopy (NIRS) (Barati et al., 2013; Chance et al., 1998; Villringer & Chance, 1997). NIRS is a non-invasive and easy-to-use modality, and with the exception of its moderate depth of penetration and spatial resolution, it provides information similar to that of fMRI. In particular NIRS has been successfully used to monitor brain response to noxious stimuli (Barati et al., 2013; Chance et al., 1998; Villringer & Chance, 1997). NIRS is as an optical brain imaging modality that implements near infrared light to track changes in cerebral hemodynamic responses. Commercially available

NIRS system can probe 2-3 centimeters into the tissue, utilizing wavelengths within the 600 to 900nm, known as the "optical light window." At approximately 700nm, deoxy-hemoglobin is absorbed at a maximum and at approximately 900nm, oxy-hemoglobin is absorbed at a maximum (Barati et al., 2013). Through modified Beer-Lambert law calculations, we obtain the changes in oxy and deoxy-hemoglobin concentration and an estimate of cerebral blood flow.

In our laboratory, near infrared spectroscopy (NIRS) was successfully used as a monitor for the hemodynamics response to cold stimuli. In these studies, subjects immersed their right hand in a bucket of ice water and the autonomic responses were monitored from the forehead by a NIRS probe (Barati et al., 2013). The correlation between hemodynamic response measured by fNIRS and cold noxious stimuli has been demonstrated through several cold pressor test (CPT) studies. (Barati et al., 2013)

Presently, we are improving this method by machine-learning techniques such as logistic regression and support-vector machine to classify the measured results taken during painful versus non-painful states as reported by the subject. We have found features in the signal that correlate to the intensity of the stimuli and were used to train a machine learning system to classify signals based on two levels of pains – low pain and high pain – with an accuracy of about 90%. Furthermore, we are planning to use other noxious stimuli that would evoke less generalized systemic responses such as pressure algometry and electrical stimulation in order to tease out the cortical response from the systemic autonomic response.

Reliable biomarkers are of great need and importance in evidence-based, personalized management of pain. fNIRS has been used as a method to objectively measure the autonomic response to noxious stimuli and has proven to be linked to subjective pain responses (Barati et al., 2013). Given this information, it has been suggested that fNIRS can be used as a potential

technique for identifying robust biomarkers for pain and individual responses to noxious stimuli. These biomarkers are essential in evidence-based pain management, as they account for the subjective nature of pain. (Barati et al., 2013)

Innovation

The diffuse optical imaging modality of functional near infrared spectroscopy (fNIRS) has been used for countless applications, both in the clinical setting and experimental setting, as a monitor of changes in body hemodynamics. These applications demonstrate the versatility of fNIRS and its usefulness as an imaging modality as compared to its more expensive lacking, although somewhat effective, counterparts such as PET, SPECT, fMRI and EEG. PET and SPECT are very similar in their use of gamma rays for 3-dimensional modeling to give high spatial resolution brain images (Borsook et al., 2010). While pain, as well as adaptation to pain, are things have been extensively researched in the pain community, studying adaptation to pain through the use of this innovative diffuse optical imaging modality of fNIRS has not. Not only this, but then comparing these fNIRS signals to subjective pain scores in the scope of adaptation has also yet to be thoroughly researched. The research conducted in fulfillment of this dissertation will help to fill this gap in research and highlight the use of fNIRS in adaptation studies. In understanding adaptation, what this yields is a greater understanding of the bodies' response to pain. For instance, if one never adapts to pain when undergoing pain testing, something is wrong with the way they adapt to pain. What this exposes is a problem in the pain pathway, identifying a need for intervention preemptively as opposed to post-injury interventional treatments. All of the previous work done in understanding pain, neuroimaging of pain, and pain perception, have all advanced pain research in a great way. What the work here will do is help demonstrate how adaptation also plays a key role in understanding pain, and how it may assist clinicians in diagnosing and treating

pain problems in the future. EEG acts as a source localization technique through which we utilize electrical signals in the brain and source localization to get information about brain activity. Lastly, fMRI, which is most similar to fNIRS, uses BOLD imaging or blood-oxygenation-level-dependent contrast imaging, but is far more costly when compared to fNIRS. As the technology has improved and been commercialized over the last 20 years since the inception of fNIRS (Boas, Elwell, Ferrari, & Taga, 2014), there has been increasing interest in using this technology for neuroimaging research (Boas et al., 2014). It has been used as a part of multiple applications, from depth of anesthesia during surgery, to assessing cognitive performance levels (Borsook et al., 2010). Using fNIRS, its applicable reach can be anticipated to extend further by being able to measure multiple pain types, by monitoring the hemodynamics in the dorsolateral prefrontal cortex region.

1.6 Outline

The subsequent chapters of this thesis are organized as follows:

Chapter 2 of this dissertation will focus on AIM 1 which focuses on the hemodynamic response to repeated exposure of stimuli. This will give a view of how the response changes over time, and the sensitivity of the fNIRS signal to observe adaptation in healthy subjects.

Chapter 3 will focus on the correlation of the fNIRS signals to standard perception measures utilized in the field of pain research. With pain being heavily subjective in nature, it is important that the signal matches subjects' subjective experience to pain. This will be accomplished primarily through using three subjective measures: (1) the visual analogue scale (VAS), (2) the Short-Form McGill Pain Questionnaire (SF-MPQ) and (3) the state trait anxiety

inventory (STAI). Looking at how the measures of perception change over time in correlation to the fNIRS signal will confirm findings in Chapter 2.

Chapter 4 will focus on the use of unsupervised machine-learning techniques in an exploratory analysis to see if the data can be grouped into categories solely based on the signal response given at different time points of the repeated exposure to stimuli. As adaptation is a diminished response to repeated exposure of a given stimuli, being able to separate the data into different groups based on time of exposure (i.e. initial exposure, final exposure) will further expand and confirm results obtained in Chapter 2 and Chapter 3. It will show the clusters that could be defined based on trial number of repeated measure experimentation.

Lastly, Chapter 5 will summarize the findings of the work done as a part of this dissertation, and suggest future work to be done in this regard.

Chapter 2

AIM 1. fNIRS and Adaptation. Explore the adaptation of healthy subjects to repeated exposure of noxious stimuli.

2.1 Introduction

As a part of the peripheral nervous system, there is the autonomic nervous system that for the most part, unconsciously controls our bodies regulatory functions (Barati et al., 2013; Ohsawa, Okada, Nishijo, & Sato, 1995). Within the autonomic nervous system there is the sympathetic nervous system which is responsible for our bodies physiological reaction to an event that is perceived as threatening or harmful in any way, also termed the "fight-or-flight" response. In experimental use of fNIRS, the unique feature that is taken advantage of is the "fight-or-flight" response. What this does is tell the body that there is some external stimulus that is being experienced by the body. In the "fight-or-flight" response, as the name indicates, the decision that is plaguing the brain at that moment is to fight the arousal away or flee from the location of the stimulus. While the brain typically experiences pain in a multitude of areas that all contribute to the pain experience (i.e. the pain matrix), this unique feature of the autonomic nervous system allows for the use of fNIRS on the forehead to take measurements that relate to a painful experience.

In this investigation, as we are focusing on adaptation, we see how the autonomic response is changed through exposure to thermal stimuli over time, specifically through using the cold pressor test (CPT). Previous studies with fNIRS and pain have been able to prove fNIRS as a way to objectively detect pain (Barati et al., 2013). In these studies, the objective was simply to understand whether fNIRS is reliable in quantifying relative pain experienced by subjects. Our study expands upon this and looks to employ a repeated measures approach to monitor the pain

response over time. Thus, the goal of the experiments conducted in the scope of this chapter are to see how reliable the hemodynamic biomarkers obtained by the fNIRS signal can assess a subjects' adaptation to repeated exposure to a stimulus over time.

2.2 Materials and Methods

2.2.1 fNIRS Principles and Instrumentation

fNIRS Imaging and Signal Processing

The fNIRS continuous-wave imaging system penetrates tissue up to 2-3cm within the 700 to 900nm optical light window depending upon the geometrical configuration of the distance between detector and LED light source (see Fig. 1) (Ferrari & Quaresima, 2012). The configuration followed in this study was done based on several preceding studies that included theoretical and experimental analysis of source-detector separation (Barati et al., 2013; Ferrari & Quaresima, 2012).

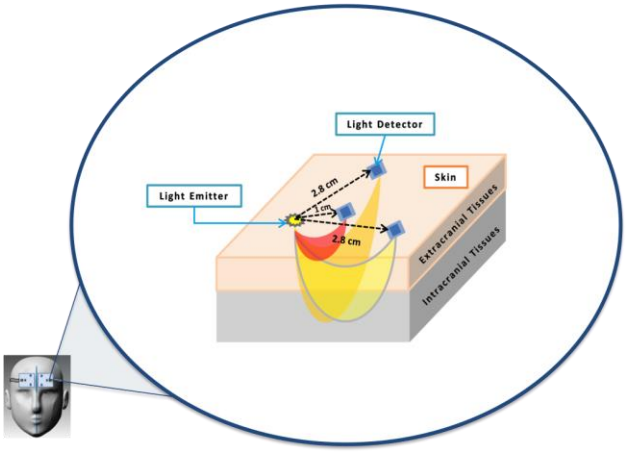


Figure 1. Schematic of probe configuration.

The fNIRS system can penetrate tissue up to a few centimeters within the 700 to 900nm optical light window. In this window, water is minimally absorbed to allow for maximum

absorption of other chromophores, primarily hemoglobin in the given light window (Barati et al., 2013). When light is emitted from the LED light source in the forehead region (see Figure 1), it is both scattered and absorbed. The detectors then detect the amount of light that reaches back to the surface of the skin due to multiple sequential scattering (Simonson & Piantadosi, 1996). The signals obtained from the detectors can be thought of as light intensity values as they are taking a measure of light scattered and light absorbed (Barati et al., 2013). Based on the changes of the amount of light absorbed as changed from baseline, through the modified beer-lambert law (see Equation in section 2.3.4 below), a ratio is obtained that gives the changes in oxy-hemoglobin (HBO_2) and deoxy-hemoglobin (HB) in the forehead region. The specifications behind the instrumentation, safety and signal to noise estimations have been studied extensively prior to commercial use of fNIRS modality in pain research (Barati et al., 2013).

Constant Temperature Bath

The CPT was used as a means of inducing acute pain from a thermal stimulus. A constant temperature bath (Lauda-Brinkmann RM6-RMS Refrigerating Circulating Bath) was used to maintain a temperature of 4°C throughout testing. This temperature has been used frequently in the literature as it induces pain, but not at an unbearable level – allowing subjects to endure for the duration of testing. (Barati et al., 2013)

2.2.2 Subjects

Healthy subjects (n=15) with no history of neurological, psychiatric or psychological disorders and who were not currently on any medications were recruited from the Drexel University community. Using the Edinburgh Handedness Inventory, subjects were confirmed to be right handed before study participation was allowed. Upon understanding all experimental procedures, subjects were required to sign the informed consent document before participating in

any further study related activities. The Drexel IRB, through the university's Human Research Protection Program, approved all study proceedings. The basis for these hypotheses are found by precedent in previously published literature by our lab (Barati et al., 2013; Pourshoghi et. al 2015).

2.2.3 Protocol

Subjects were seated comfortably with minimal distractions for the duration of experimentation. All data recording was done in a dim environment to prevent the effect of ambient light on data recording. Subjects each had two probes on their forehead, each of which had 3 detectors. Channels 1, 2 and 3 were on the right-side probe and channels 4, 5 and 6 were on the left-side probe. These were used to monitor the subjects' hemodynamic response during experimentation. Each experiment consisted of 7 trials of the experimental procedure post the 30-second baseline recording. The procedure included placing the right hand in tepid water (maintained at 23°C) for 2 minutes, followed by immersion of the hand in 4°C water for 30 seconds. After 7 trials, the subject then placed their hand in tepid water for 2 minutes to conclude the experiment. All subjects maintained appropriate timing by following given auditory commands from the experimenter. (Barati et al., 2013) This is seen in Figure 2.

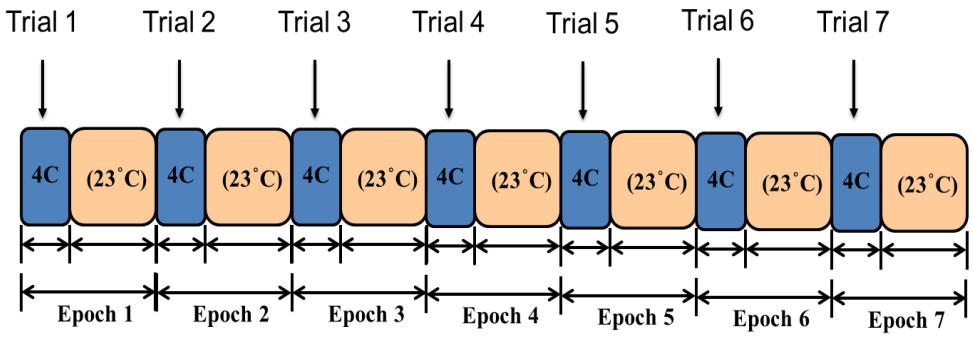


Figure 2. Repeated measures protocol diagram.

2.2.4 fNIR Data Processing

Equation 1. Modified Beer-Lambert Law

$$\Delta \mathrm{OD}_{\lambda} = \log\left(\frac{I_{\mathrm{b}}}{I_{\mathrm{t}}}\right) = \varepsilon_{\lambda}^{\mathrm{Hb}} \Delta c^{\mathrm{Hb}} \mathrm{d}\, \mathrm{DPF}_{\lambda} + \varepsilon_{\lambda}^{\mathrm{HbO_2}} \Delta c^{\mathrm{HbO_2}} \mathrm{d}\, \mathrm{DPF}_{\lambda}$$

$\Delta OD\lambda$ is termed the optical density. I_b is the light intensity during baseline and I_t is the baseline after a given task has been performed. $\varepsilon_{\lambda}^{Hb}$ and $\varepsilon_{\lambda}^{HbO_2}$ are the absorption coefficients for the hemoglobin molecules at wavelength lambda. Δc^{Hb} and Δc^{HbO2} represent concentration changes in the hemoglobin molecules due to the given task. 'd' represents the distance between the light source and photodetector. DPF_{λ} is the differential pathlength factor adjusted for the increased pathlength from source to detector due to scattering at the wavelength lambda. When measured at two different wavelengths, this equation can then be solved for the change in the concentration of HB and HBO2 molecules.

Data Collection

The far detectors (distance of 2.8cm from the light source) were used in analysis post-experimentation. This is a common experimental practice in the use of fNIRS as the near detector (1cm from light source) only obtains information from the superficial layers while the far detector penetrates into the cortical tissue, giving information on the brain's response to stimulation.

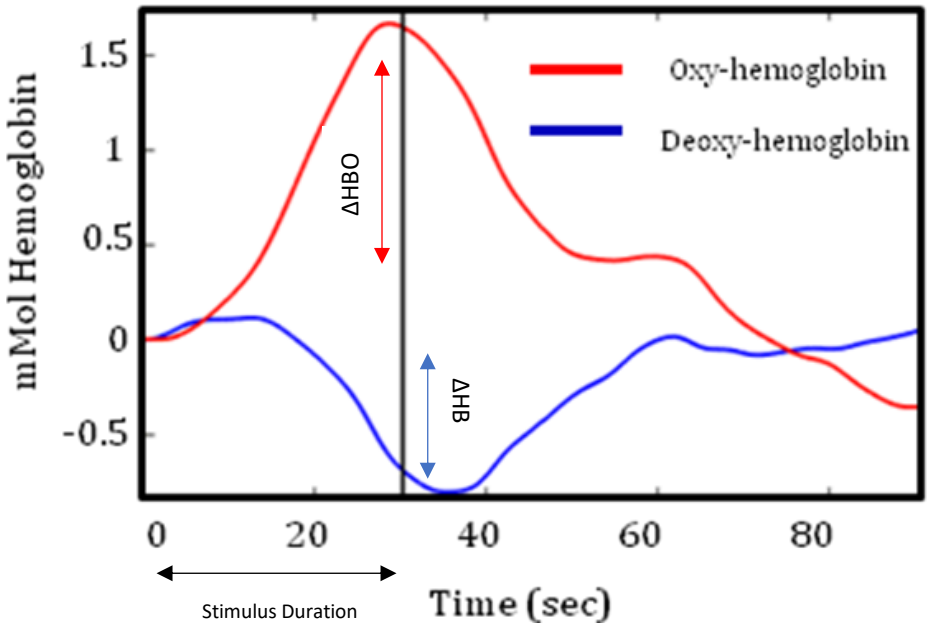


Figure 3. Example near infrared spectroscopy signal of oxy and deoxyhemoglobin.

Preprocessing of Data

Data was collected at a sampling frequency of 2Hz and analyzed through the use of MATLAB (R2015a, MathWorks, Natwick, MA). Smoothing techniques were performed through using the functional data analysis (fDA) package in MATLAB, coupled with a finite impulse response low-pass filter with a cutoff frequency of 0.14Hz as determined based on previous fNIRS studies (Barati et al., 2013). This helped to reduce noise and motion artifact, as well as heart rate and respiration influence on the signals (Barati et al., 2013). HbO2 and HHb concentrations were calculated in comparison to their baseline optical intensity values that were obtained in the pre-stimulus period, which consists of a 30-second baseline period. Bspline basis expansion is used to impose a penalty on roughness of the second derivative of the data with lambda values of 300; the total hemoglobin is calculated in addition to the concentrations of oxy and deoxy-hemoglobin

were calculated in relation to their baseline optical intensity values obtained in the pre-stimulus 30-second baseline period. See Figure 3 for an example of what this processing produces.

Statistical Analysis

With univariate repeated measures ANOVA, time is considered as a categorical variable (Tak & Ye, 2014). This allows for comparisons of one time point to another (e.g. comparing trial 1 to trial 3, trial 1 to trial 3, etc.). Therefore, multilevel modeling (MLM) growth or decay models proved useful in this analysis, as time is considered a continuous variable. This allows for observations of changes over time (e.g. slope from trial 1 through trial 7, the significance of the slope, etc.). It also accounts for any unequal spacing between time intervals and unbalanced data, and can increase the statistical power of detecting growth or decay effects. (Kwok et al., 2008)

Another considered approach is linear regression; however, compared to this mixed model approach, regression requires that observations are independent of one another. Here, we have repeated measures and thus, this assumption is violated so we cannot use regular regression. With multilevel modeling being hierarchical in nature, it allows for random intercepts and slopes for different subjects (Kwok et al., 2008). These rationales highlight why multilevel modeling was used in the case of the repeated measures cold pressor test employed in this chapter. A summary of results can be found in tables 1 through 4, with graphical representations of the results of each channel displayed in Figures 4 through 27. Significance of change is identified in this chapter by the following designations: *=Significance of $p< 0.10$, **= Significance of $p <0.05$, and ***=Significance of $p < 0.01$.

2.3 Results

2.3.1 Multilevel Modeling of Deoxyhemoglobin for Trial 1 through Trial 7

In using multilevel modeling for deoxyhemoglobin, the best model used for channels 1 through 6 allowed the intercept to vary across subjects. When looking at deoxyhemoglobin for channel 1, a near channel, what we see is that over time (trials 1 through 7), we see that 4°C starts with a ΔHB of 0.062 at trial 1, with a negative slope of -0.024 over time to trial 7. The results are shown in Figure 4; there was no significant change to ΔHB over time. In 15°C we see similar results with a starting point of -0.145 for change in deoxyhemoglobin and a small slope of -0.001. Also, show in in Figure 4, the results for 15°C do not show a significant change in ΔHB over time.

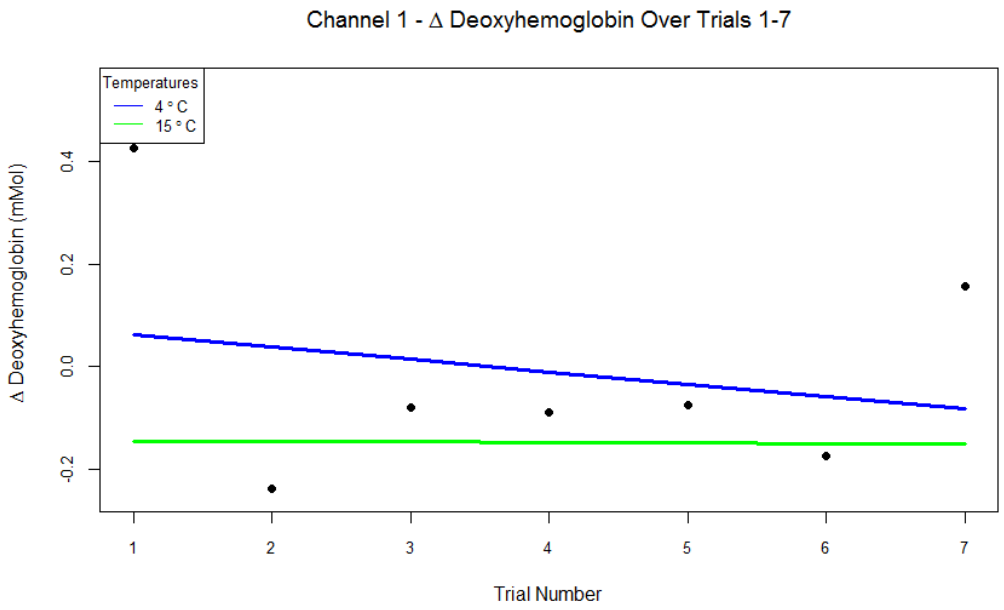


Figure 4. Channel 1 (near channel – 1cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

For channel 2, we see that 4°C starts with a ΔHB of -0.391 at the first trial and a positive slope of 0.037 over time through to trial 7. These results are shown in Figure 5 with no significant change in ΔHB over time. Similarly, for 15°C, the grand mean at trial 1 is -0.119 and a negative slope of -0.008 as it moves through to trial 7. These results are also shown in Figure 5 and demonstrate no significance in the ΔHB over time.

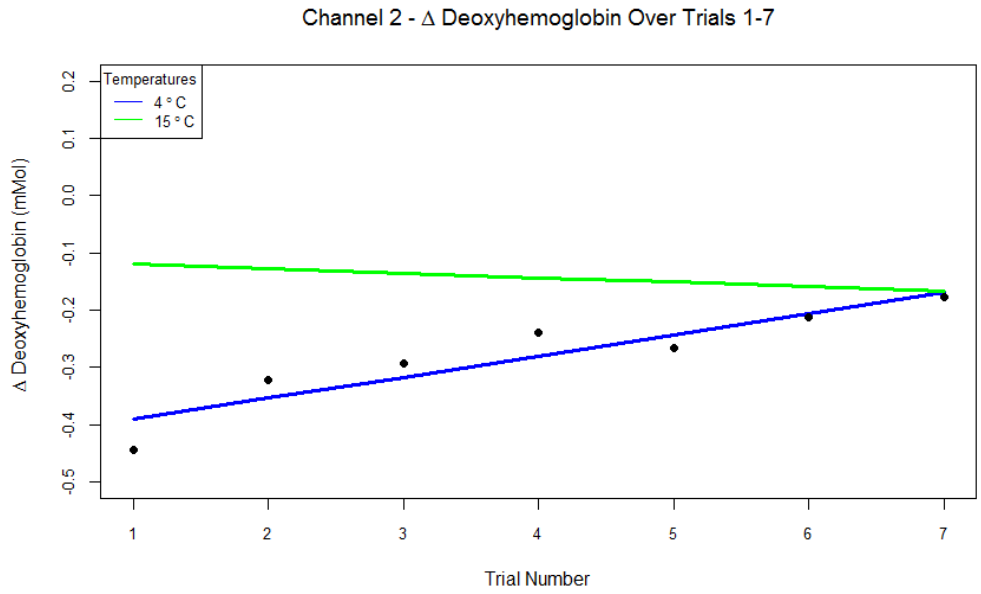


Figure 5. Channel 2 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

When looking at the results for channel 3, 4°C shows a ΔHB of -0.362 during the initial exposure with a slope of 0.047; these results were significant with a p-value of 0.044. This shows that the change in deoxyhemoglobin significantly changed over time in the case of channel 3 at 4°C; whereas, in the case of 15°C, with a starting point of 0.001 for change ΔHB, and a small negative slope of -0.012, there was no significant change in ΔHB over time from trial 1 through trial 7. These results are shown in Figure 6.

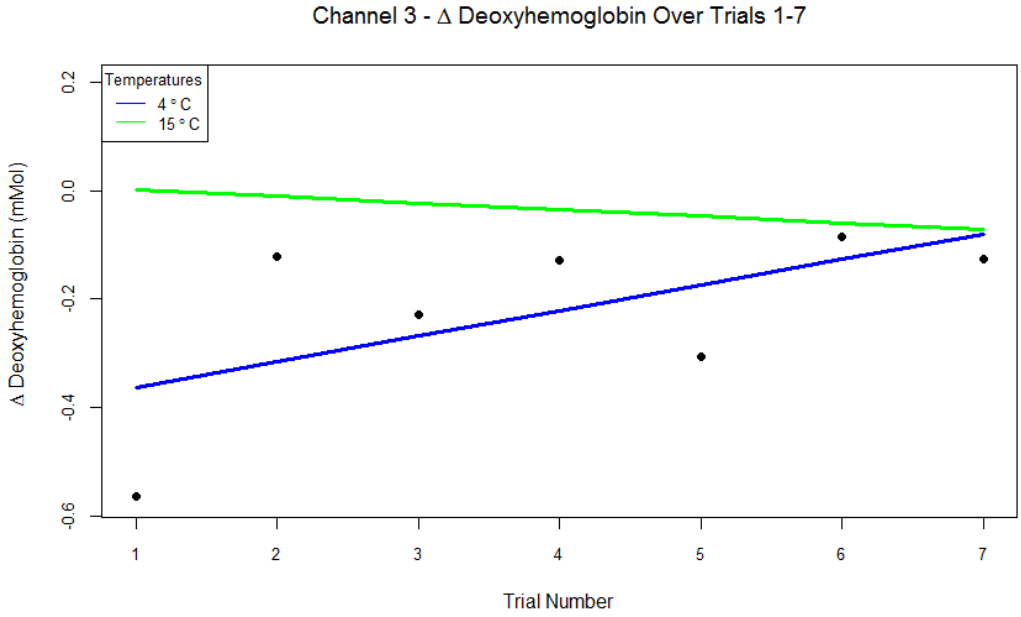


Figure 6. Channel 3 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

ΔHB for channel 4 at 4°C shows a trial 1 grand mean of -0.222 with a slope of 0.075, that showed a significant increase over time with a p-value of 0.039 when looking at the change over time from trial 1 through trial 7. When comparing this to 15°C, as demonstrated in Figure 7, there was, in contrast, no significant change over time, with a trial 1 grand mean of -0.150 and a minimal slope of 0.006.

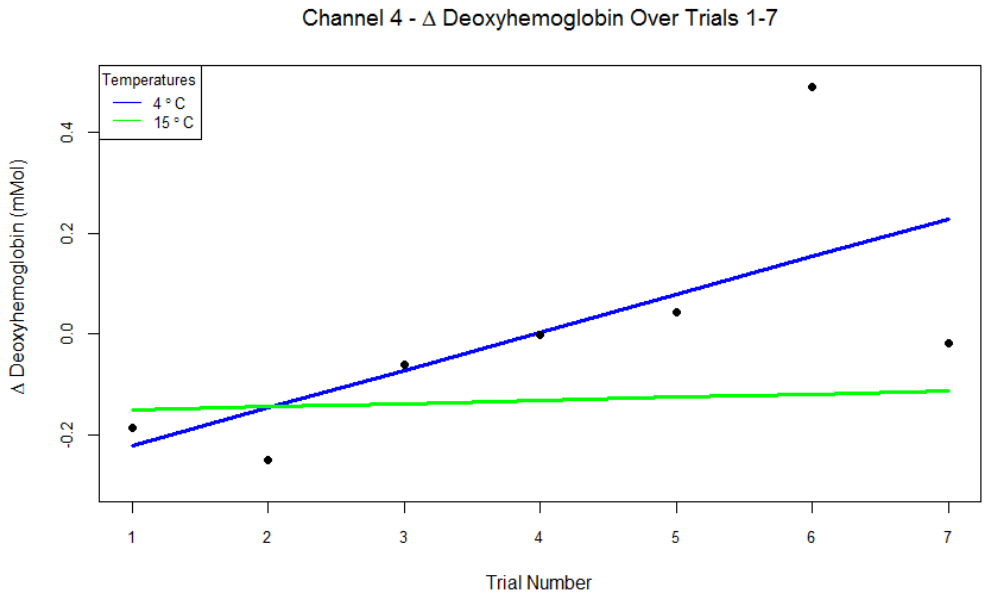


Figure 7. Channel 4 (near channel – 1cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

For channels 5 and 6 at 4°C, the trial 1 grand means for ΔHB were -0.418 and -0.356 respectively with matching slopes of 0.077 and 0.067. Both positive slopes showed a significant change over time from trials 1 through trial 7 (4°C at channel 5 p-value: 0.003; 4°C channel 6 p-value: 0.003). Lastly, for ΔHB at 15°C, the grand means for trial 1 of -0.115 and 0.009 and slopes of 0.003 and -0.009 respectively did not show any significant change over time (15°C at channel 5 p-value: 0.897; 15°C channel 6 p-value: 0.763). These results are shown in Figure 8 and Figure 9.

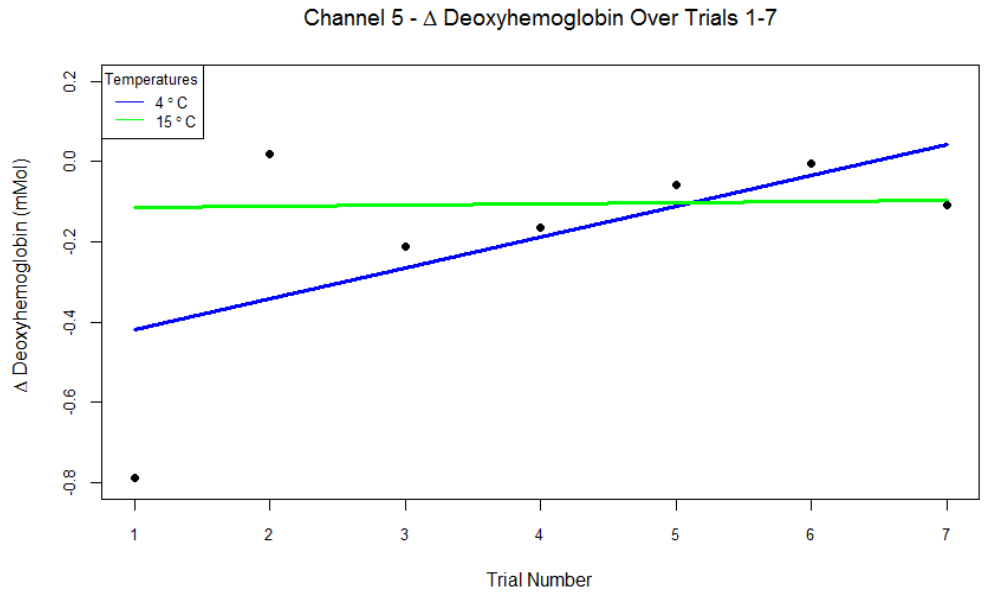


Figure 8. Channel 5 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

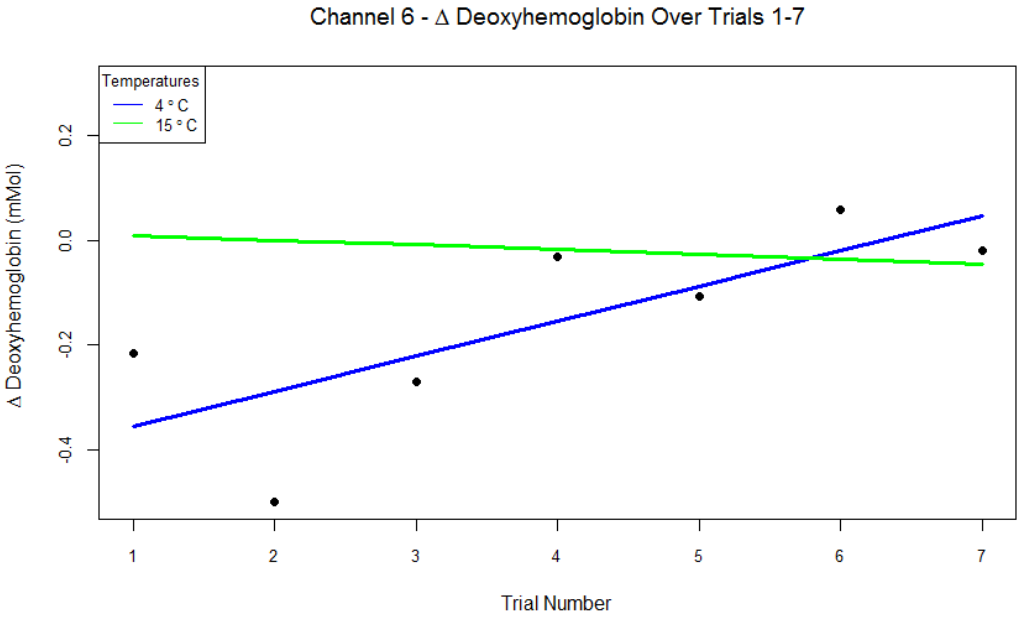


Figure 9. Channel 6 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHB at each trial for 4 degrees Celsius.

Channels	Temperature	Trial 1 (grand mean)	Slope	p-Value
1 (1cm)	4˚C	0.062	-0.024	0.846
	15˚C	-0.145	-0.001	0.984
2 (2.8cm)	4˚C	-0.391	0.037	0.258
	15˚C	-0.119	-0.008	0.591
3 (2.8cm)	4˚C	-0.362	0.047	0.044**
	15˚C	0.001	-0.012	0.461
4 (1cm)	4˚C	-0.222	0.075	0.039**
	15˚C	-0.150	0.006	0.833
5 (2.8cm)	4˚C	-0.418	0.077	0.003***
	15˚C	-0.115	0.003	0.897
6 (2.8cm)	4˚C	-0.356	0.067	0.003***
	15˚C	0.009	-0.009	0.763

Table 1. (Deoxyhemoglobin) Numerical results from Figure 4 through Figure 9 showing the grand mean for trial 1, the representative slope for the model fitting, followed by the significance value (*=Significance of p< 0.10, **= Significance of p <0.05, and ***=Significance of p < 0.01).

2.3.2 Multilevel Modeling of Deoxyhemoglobin for Trial 2 through Trial 7

Now we can move to the case of Trials 2 through 7. In this case, as trial 1 proved to be the most significant in that initial exposure causes the greatest disturbance to the system. As a result, we have removed trial 1 from the analysis of deoxyhemoglobin in this section and are solely looking at trials 2 through 7. For all figures in this section (Figure 10 through Figure 15), all figures with trial numbers numbered 1 through 6 refers to the order of the trial and not the actual trial number (i.e. trial 1 is the first trial, so trial 2, trial 2 is the second trial, so trial 3, etc.).

For ΔHB in channel 1 (see Figure 10) when looking at 4°C and 15°C, the first trial (trial 2) grand mean is -0.205 and -0.158, with slopes of 0.035 and 0.003 respectively. The p-value for 4°C is 0.147 and for 15°C is 0.933, showing that for ΔHB there is no significant change over time when removing the initial exposure from the analysis. In this section, the average of ΔHB was not shown represented as dots on each graph as the results for this would be the same as in the previous section.

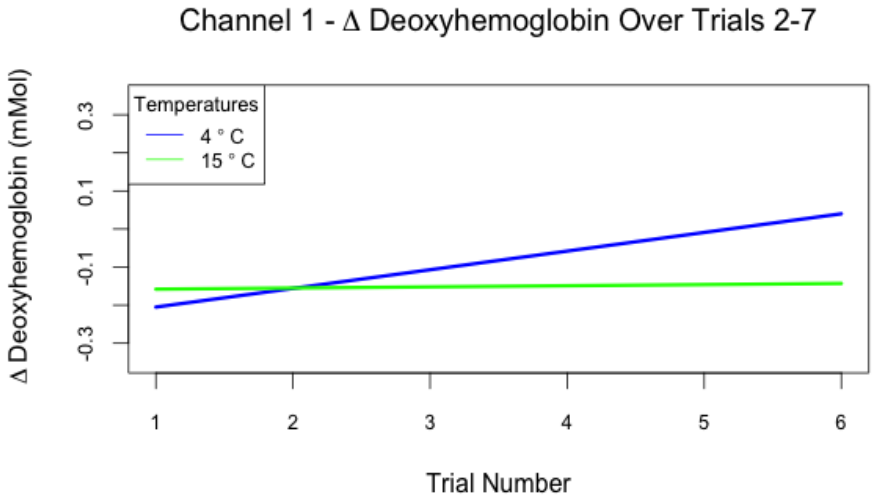


Figure 10. Channel 1 (near channel – 1cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

When considering channels 2 and 3 (both far channels on the right side), the trial 2 grand means for ΔHB were -0.319 and -0.182 for 4°C and -0.307 and 0.037 for 15°C. Upon further analysis, there slopes were 0.027 and 0.007, and -0.014 and -0.026 respectively. Overall, regardless of temperature, in channels 2 and 3, there was no significance observed over time from trial 2 through 6 when removing the initial exposure to the thermal stimuli. Summaries of this data can be viewed in Figures 11 and 12; details of results for channels 2 and 3 can be seen in Table 2.

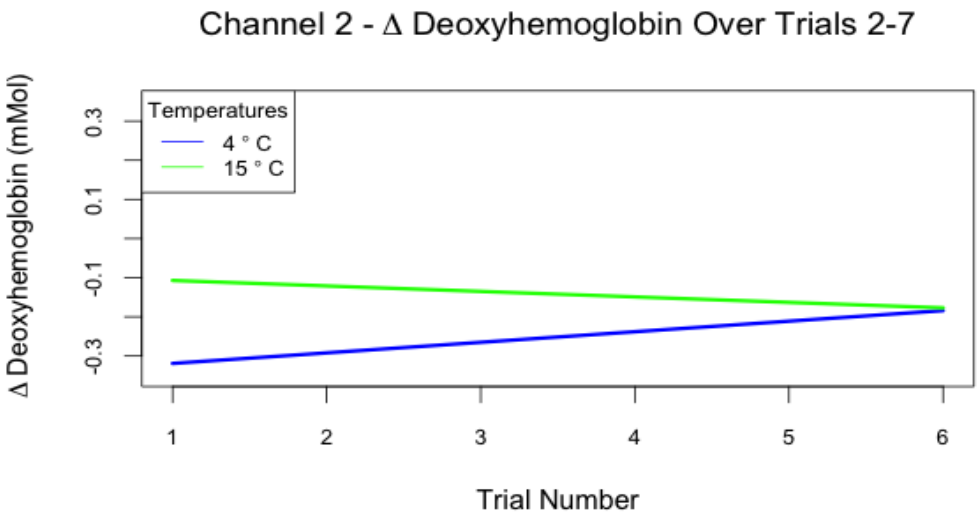


Figure 11. Channel 2 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

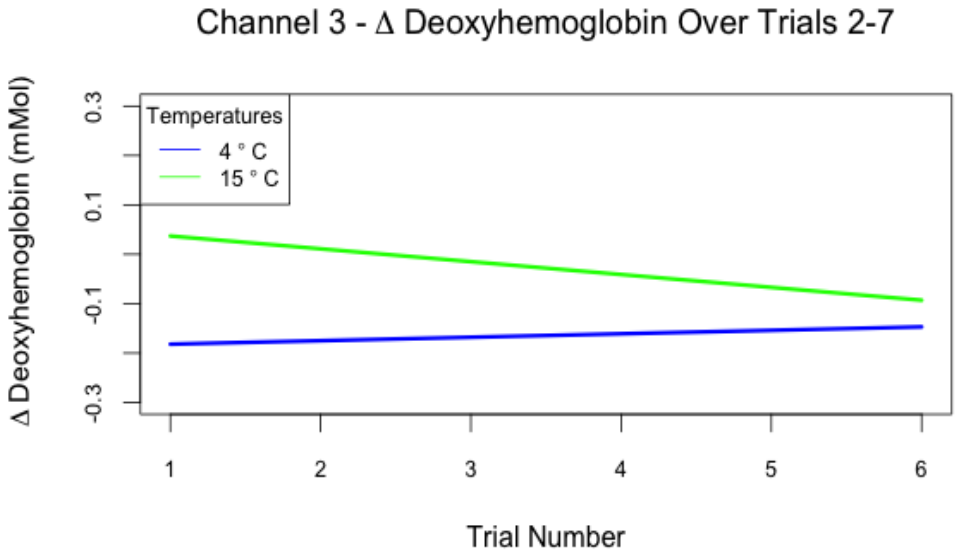


Figure 12. Channel 3 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

Details for channels 4 and 5 can be found in Table 2, and seen graphically in Figures 13 and 14; however, they are similar to results found in channels 1, 2 and 3 in that the grand means in conjunction with the small slopes when modeling ΔHB, do not demonstrate a significant change over time (when looking solely at trials 2 through 6 after removing the initial exposure from analysis).

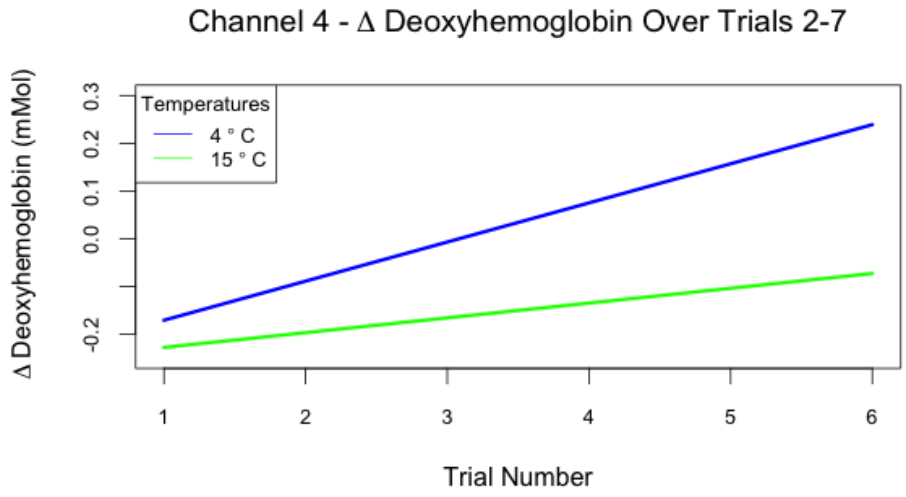


Figure 13. Channel 4 (near channel – 1cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

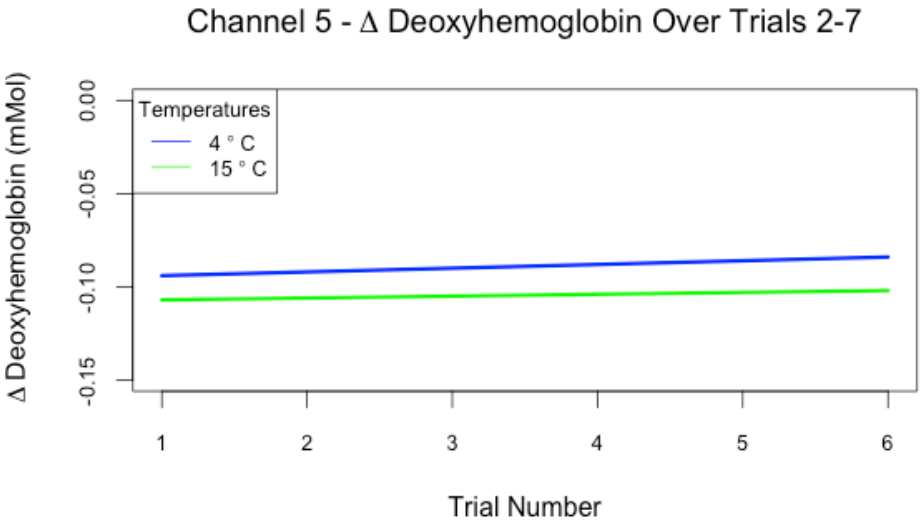


Figure 14. Channel 5 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

Lastly, when looking at the channel 6 data for ΔHB at 4°C, the grand mean for the first trial (trial 2) is -0.381 with a slope of 0.094. The resulting p-value from this is 0.004 which shows that there was a significant increase over time in ΔHB when looking at trials 2 through 7. When looking at the channel 6 data for ΔHB at 15°C, the grand mean for the first trial is -0.038 with a slope of 0.002, yielding a p-value of 0.954; thus, there was no significant change over time in the case of channel 6 at 15°C. This can be seen graphically in Figure 15.

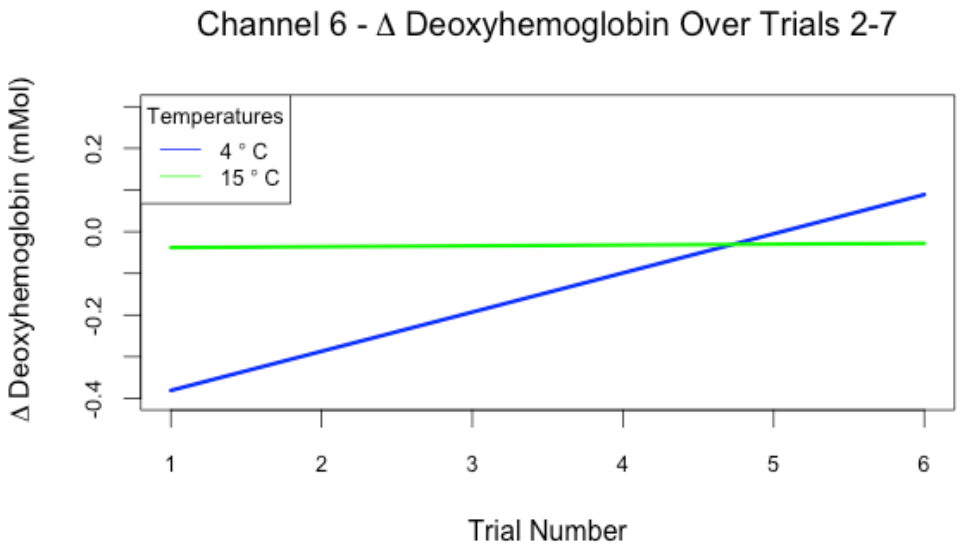


Figure 15. Channel 6 (far channel – 2.8cm) comparison of the change in deoxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

Channels	Temperature	Trial 2 (grand mean)	Slope	p-Value
1 (1cm)	4˚C	-0.205	0.035	0.147
	15˚C	-0.158	0.003	0.933
2 (2.8cm)	4˚C	-0.319	0.027	0.150
	15˚C	-0.107	-0.014	0.428
3 (2.8cm)	4˚C	-0.182	0.007	0.853
	15˚C	0.037	-0.026	0.198
4 (1cm)	4˚C	-0.171	0.082	0.084
	15˚C	-0.228	0.031	0.374
5 (2.8cm)	4˚C	-0.094	0.002	0.909
	15˚C	-0.107	0.001	0.974
6 (2.8cm)	4˚C	-0.381	0.094	0.004***
	15˚C	-0.038	0.002	0.954

Table 2. (Deoxyhemoglobin) Numerical results from Figure 10 through Figure 15 showing the grand mean for trial 2 (shown graphically as trial number 1 as it is overall trial number 2 but the first trial in this analysis), the representative slope for the model fitting, followed by the significance value (*=Significance of p< 0.10, **= Significance of p <0.05, and ***=Significance of p < 0.01).

2.3.3 Multilevel Modeling of Oxyhemoglobin for Trial 1 through Trial 7

In using multilevel modeling for oxyhemoglobin, the best model used for channels 1 through 6 allowed the intercept to vary across subjects. When looking at oxyhemoglobin for channel 1, a near channel, what we see is that over time (trials 1 through 7), we see that 4°C starts with a change in the ΔHBO_2 of 0.038 at trial 1, with a positive slope of 0.118 over time to trial 7. The results are shown in Figure 16; there was no significant change to ΔHBO_2 over time. In 15°C we see similar results with a starting point of -0.124 for change in oxyhemoglobin and a small slope of 0.058. Also, show in in Figure 16, the results for 15°C do not show a significant change in ΔHBO_2 over time. Further details of this are outlined in Table 3.

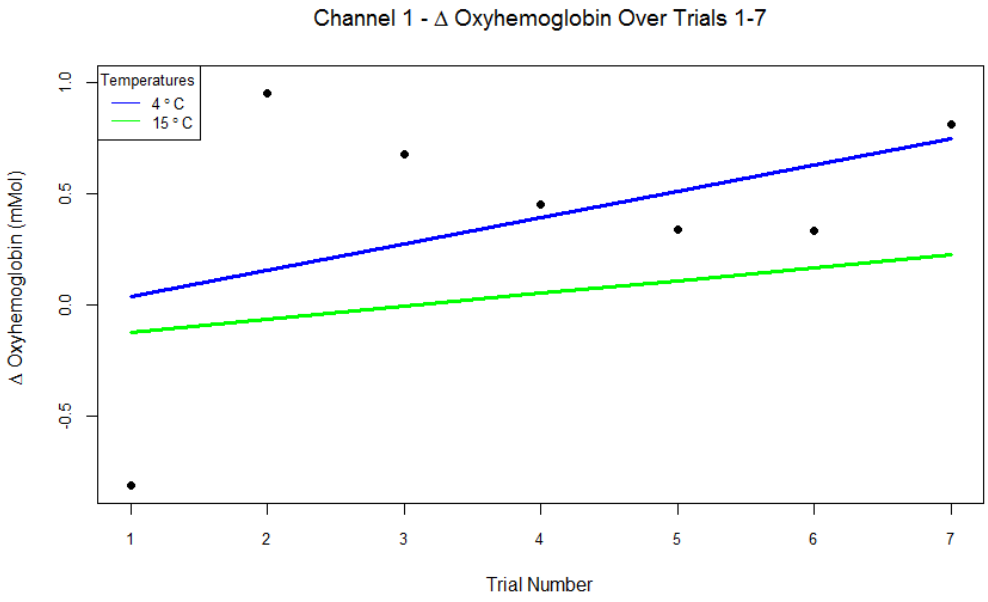


Figure 16. Channel 1 (near channel – 1cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

For channel 2, we see that 4°C starts with a ΔHBO_2 of 2.101 at the first trial and a negative slope of -0.280 over time through to trial 7. These results are shown in Figure 17 with a

significant change in ΔHBO_2 over time with a p-value of 0.001. Similarly, for 15°C, the grand mean at trial 1 is 0.008 and a positive slope of 0.013 as it moves through to trial 7. These results are also shown in Figure 17 and demonstrate no significance in the ΔHBO_2 over time.

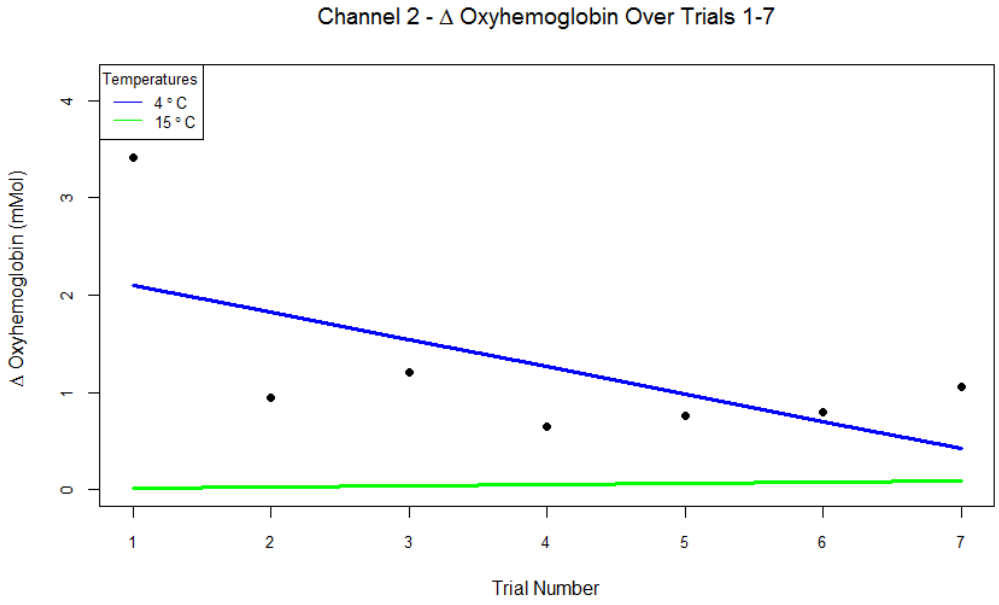


Figure 17. Channel 2 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

When looking at channels 3 and 4 for ΔHBO_2, the only case that showed significance is that of channel 3 at 4°C with a trial 1 grand mean of 1.811 and a slope of -0.256 with a p-value of 0.004 – demonstrating a significant reduction in ΔHBO_2 over time. The remaining cases (channel 3 15°C, and channel 4 4°C and 15°C all did not show significant changes over time for ΔHBO_2; results detailing this are shown in Table 3 and graphically represented in Figures 18 and 19.

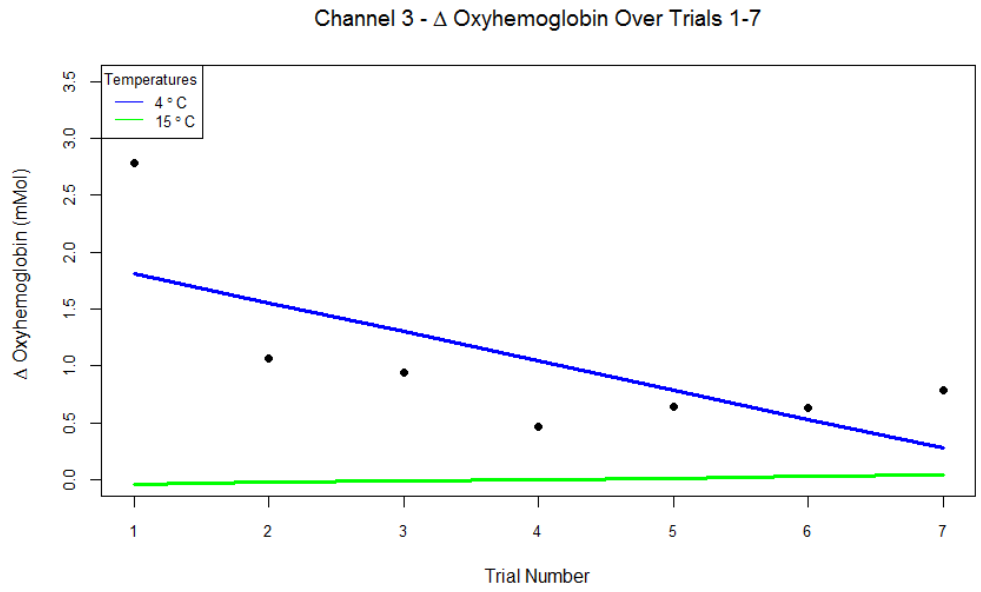


Figure 18. Channel 3 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

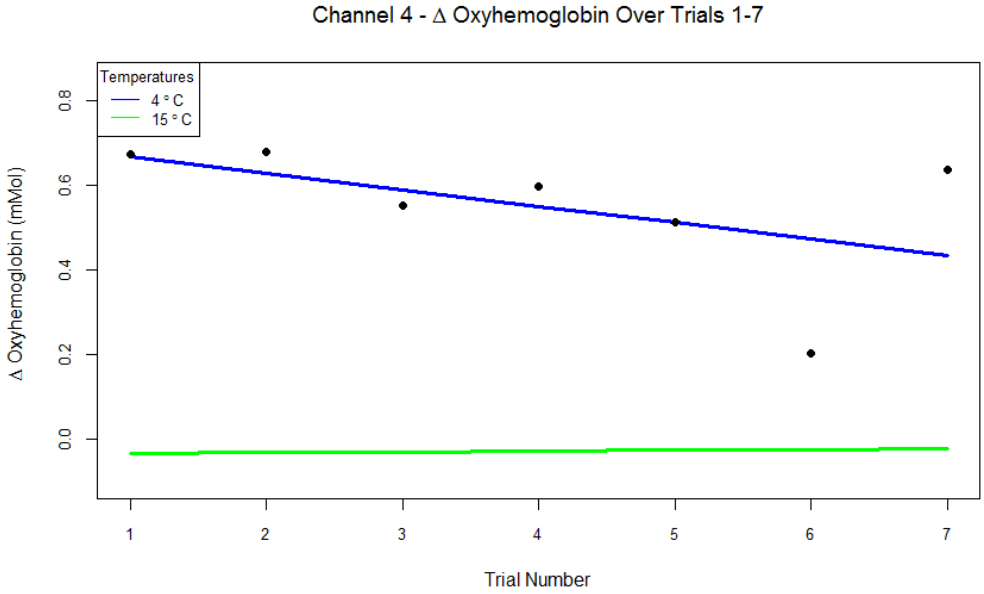


Figure 19. Channel 4 (near channel – 1cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

In the case of channels 5 and 6 (both far channels monitoring hemoglobin on the left side of the forehead), both channels showed a significant difference. Channel 5 at 4°C with a trial 1 grand mean of 1.044 and a negative slope of -0.186 with a p-value of 3.080E-6, and channel 6 at 4°C with a trial 1 grand mean of 1.137 and a negative slope of -0.243 with a p-value of 2.701E-5 – demonstrating a significant decrease in ΔHBO_2 over time. All other cases did not show significant changes over time for ΔHBO_2; these results can also be found in Table 3 and are represented visually in the graphs in Figures 20 and 21.

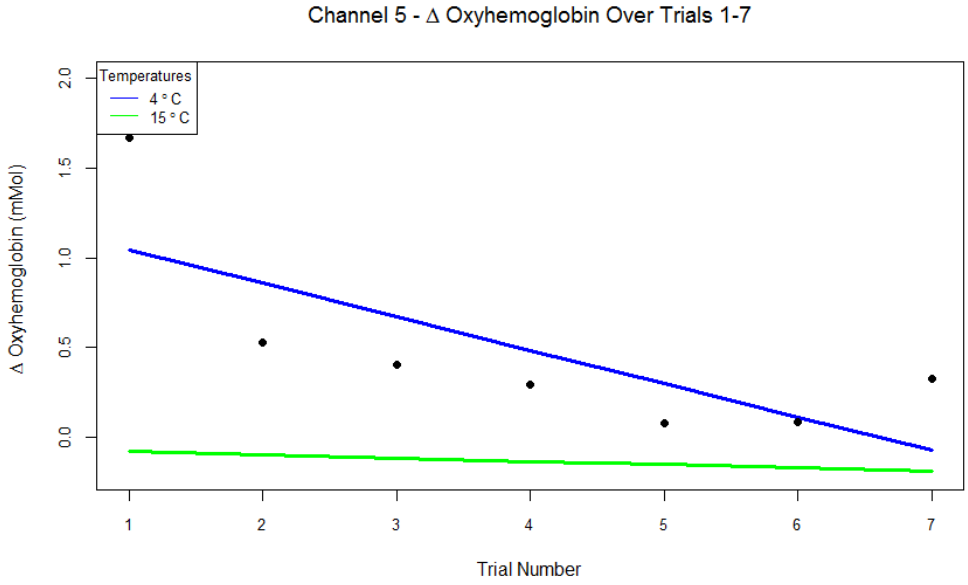


Figure 20. Channel 5 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

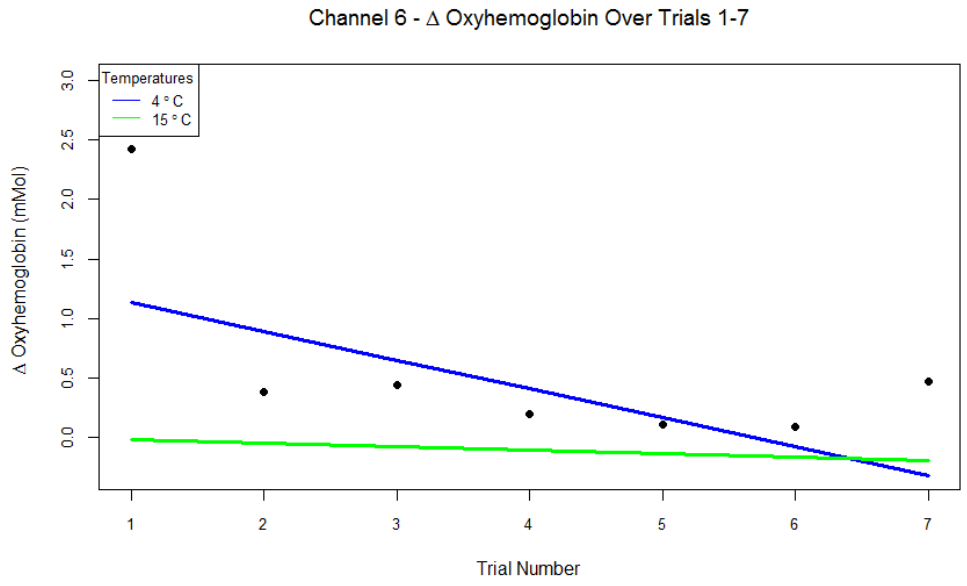


Figure 21. Channel 6 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 1 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. Black dots represent the average of all subjects' ΔHBO_2 at each trial for 4 degrees Celsius.

Channels	Temperature	Trial 1 (grand mean)	Slope	p-Value
1 (1cm)	4˚C	0.038	0.118	0.702
	15˚C	-0.124	0.058	0.137
2 (2.8cm)	4˚C	2.101	-0.280	0.001***
	15˚C	0.008	0.013	0.768
3 (2.8cm)	4˚C	1.811	-0.256	0.004***
	15˚C	-0.036	0.013	0.727
4 (1cm)	4˚C	0.667	-0.039	0.436
	15˚C	-0.033	0.002	0.963
5 (2.8cm)	4˚C	1.044	-0.186	3.080E-6***
	15˚C	-0.078	-0.018	0.650
6 (2.8cm)	4˚C	1.137	-0.243	2.701E-5***
	15˚C	-0.016	-0.129	0.741

Table 3. (Oxyhemoglobin) Numerical results from Figure 16 through Figure 21 showing the grand mean for trial 2, the representative slope for the model fitting, followed by the significance value (*=Significance of p< 0.10, **= Significance of p <0.05, and ***=Significance of p < 0.01).

2.3.4 Multilevel Modeling of Oxyhemoglobin for Trial 2 through Trial 7

Lastly, as was done in section 2.4.4 of this chapter, we can move to the case of Trials 2 through 7. This resulted in findings similar to what was done with deoxyhemoglobin, we have removed trial 1 from the analysis of oxyhemoglobin in this section and are solely looking at trials 2 through 7. For all figures in this section (Figure 22 through Figure 27), all figures with trial numbers numbered 1 through 6 refers to the order of the trial and not the actual trial number (i.e. trial 1 is the first trial, so trial 2, trial 2 is the second trial, so trial 3, etc.).

For all cases in ΔHBO_2 when looking at trials 2 through 6 for channels 1 through 6, regardless of channel or temperature, there was no significance demonstrated in terms of the changes in ΔHBO_2 over time when the initial exposure to the thermal stimuli was removed from analysis. These results can be seen in Figures 22 through 27 and are detailed in Table 4. In this section, the average of ΔHBO_2 was not shown represented as dots on each graph as the results for this would be the same as in the previous section.

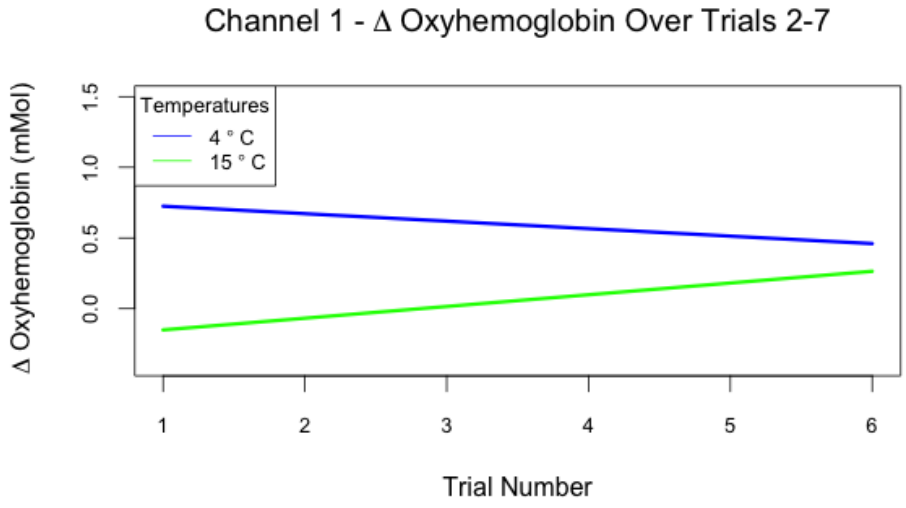


Figure 22. Channel 1 (near channel – 1cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

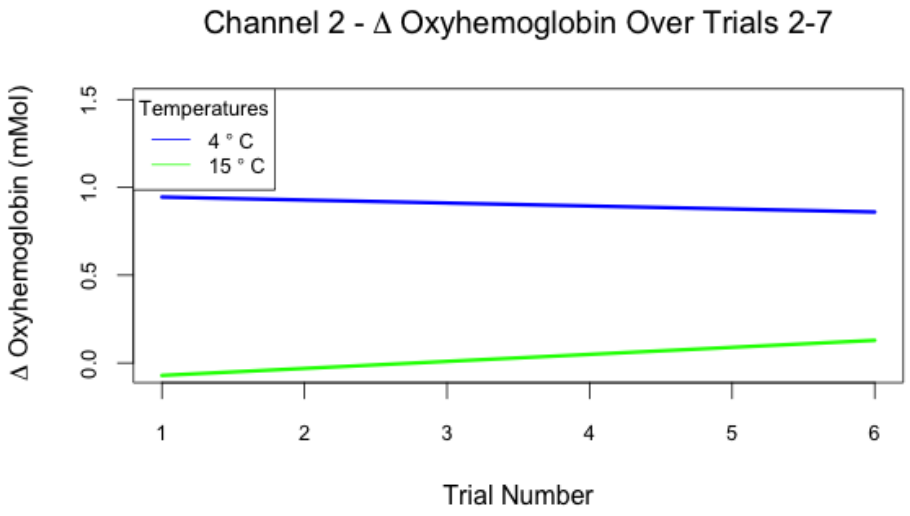


Figure 23. Channel 2 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

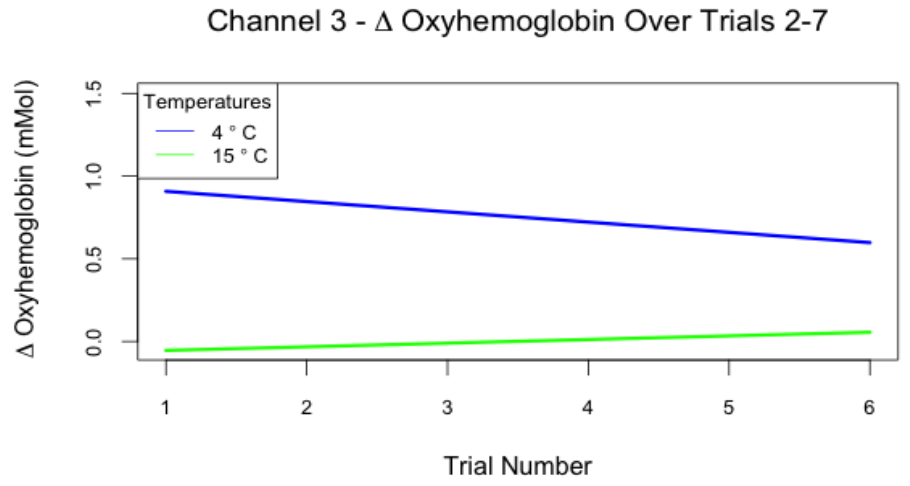


Figure 24. Channel 3 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

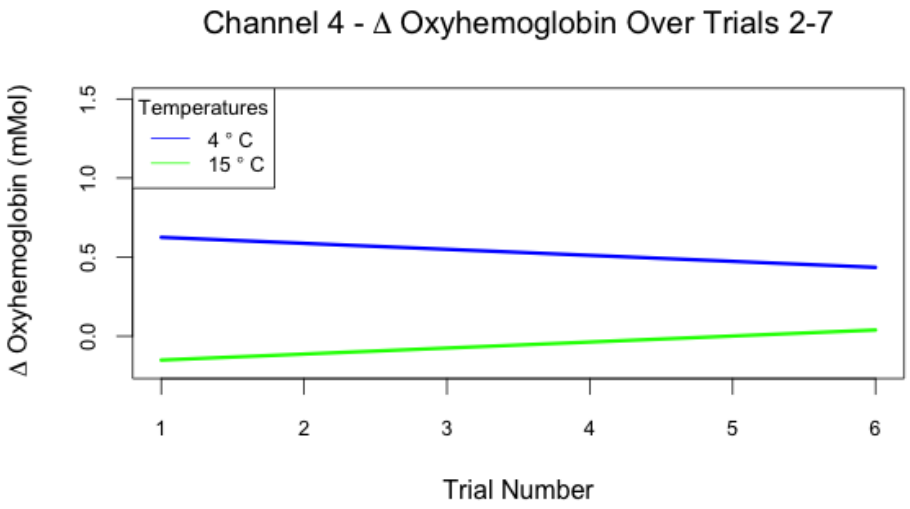


Figure 25. Channel 4 (near channel – 1cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

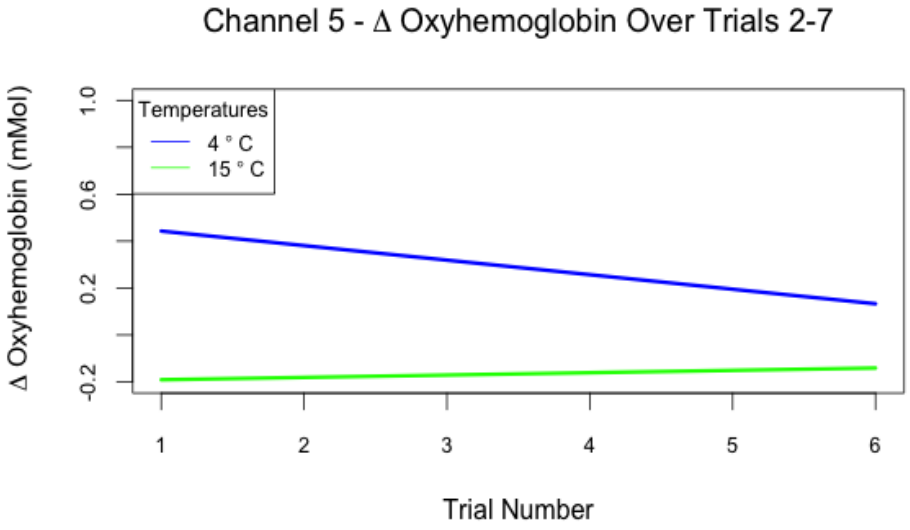


Figure 26. Channel 5 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

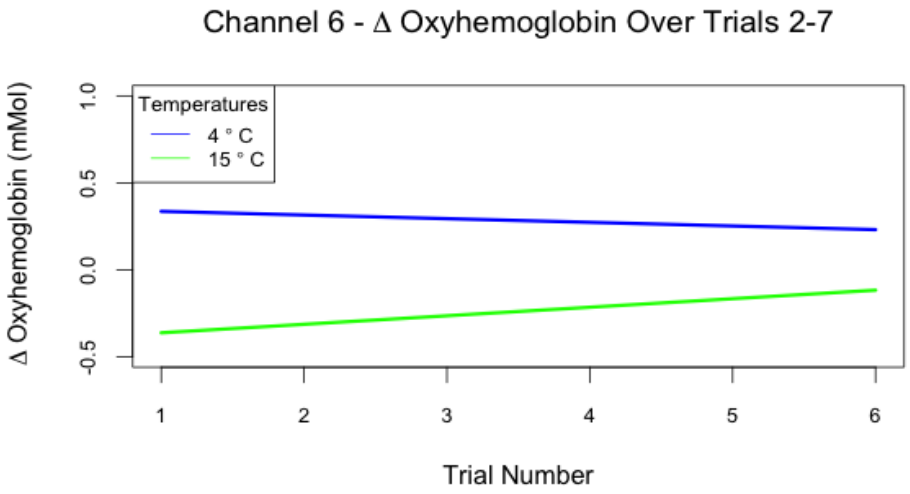


Figure 27. Channel 6 (far channel – 2.8cm) comparison of the change in oxyhemoglobin over time from trial 2 through trial 7 using multilevel modeling to estimate a slope for rate of change for both 4 degrees Celsius and 15 degrees Celsius. On the x-axis, trial number is representative of the trial order and not of the trials used in analysis.

Channels	Temperature	Trial 2 (grand mean)	Slope	p-Value
1 (1cm)	4˚C	0.725	-0.053	0.223
	15˚C	-0.151	0.838	0.075
2 (2.8cm)	4˚C	0.945	-0.017	0.704
	15˚C	-0.071	0.040	0.456
3 (2.8cm)	4˚C	0.908	-0.062	0.245
	15˚C	-0.054	0.022	0.641
4 (1cm)	4˚C	0.625	-0.038	0.346
	15˚C	-0.151	0.038	0.540
5 (2.8cm)	4˚C	0.443	-0.062	0.106
	15˚C	-0.191	0.010	0.838
6 (2.8cm)	4˚C	0.336	-0.021	0.636
	15˚C	-0.362	0.049	0.441

Table 4. (Oxyhemoglobin) Numerical results from Figure 22 through Figure 27 showing the grand mean for trial 2 (shown graphically as trial number 1 as it is overall trial number 2 but the first trial in this analysis), the representative slope for the model fitting, followed by the significance value (*=Significance of $p< 0.10$, **= Significance of $p <0.05$, and ***=Significance of $p < 0.01$).

2.4 Discussion

In performing the analysis on the data over trials 1 through 7, we met the original goal of this investigation which was simply to observe adaptation with functional near infrared spectroscopy. We see that multilevel modeling provided a great perspective on how the hemodynamic response to experimental pain changed over time using fNIRS. While the near channels (channel 1 and 4 detectors at approximately 1cm from the light source) do not show consistency, as was expected when observing superficial tissue layers, the far channels overall show a consistent reduction in the change in hemodynamic response, the more times a subject is exposed to the thermal external stimuli. More specifically, we see that channel 5 consistently showed great significance when looking at change over time. This reduction in the change of response follows the definition of adaptation, however, in the context of the fNIRS signal. The reason behind these findings in the far channels (detectors at 2.8cm away from the light source) is because with detectors at this distance, the light penetrates to a depth that reaches cortical tissues. At this depth, the hemodynamics monitored in this region are more related to activity that results from the autonomic response generated by stimuli. Given these results, we achieved the first hypothesis outlined in AIM 1 in that adaptation showed through a diminished response in the signal over time. Although not all cases were significant, what the results suggest are further investigation into adaptation (i.e. more trials during experimentation).

Upon removing trial 1 from the analysis and only looking at trial 2 through 6, we see that trial 1 is heavily influential when looking at rate of change over time. The rationale behind the removal of trial 1 is due to several other factors that influence the initial exposure to a stimulus, which can be considered the novelty of the initial exposure to experimental pain. In subsequent trials, this factor is removed and primarily where adaptation takes place; however, without the

initial exposure, there would be no stimulus to adapt to. This is seen in the data because when excluding trial 1 from analysis, the change over time was minimal regarding both Oxy and Deoxyhemoglobin. What this suggests is that the initial exposure to a stimulus initiates a response that causes a great change in the hemodynamics monitored in the prefrontal cortex region. (Barati et al., 2013) As there is no significance seen when only looking at trials 2 through 6, this suggests that from the initial exposure to the first repeated exposure (second trial), the greatest amount of adaptation occurs; however, further investigation would need to be done to confirm these findings. Following this, while a level of adaptation may still occur, it is minimal as there is no longer a great disturbance to the system, creating a situation where the body finds no need to adapt – fine tuning its response to re-achieve a level of stability.

What this may represent is the bodies' ability to adapt to external stimuli to the system, in an effort to not remain in a heightened state of "fight or flight", which would give its own set of problems as the body is not meant to constantly remain in such a heightened state. (Romero, Dickens, & Cyr, 2009) In general, to remain healthy, the body constantly strives for a level of homeostasis that keeps the bodies systems in order and functioning efficiently. The autonomic response often changes the bodies focus. The area of focus is shifted to where the external stimuli was applied, and often until the body can react appropriately and account for the given stimuli (adapt), the body does not return to its healthy state.

Further, we also learned that the rate of change between 4°C and 15°C differs significantly and that subjects demonstrated a greater rate of change over time to 4°C than they did to 15°C; or, the magnitude of the hemodynamic response decreased significantly over time when looking at 4°C and did not greatly change when looking at 15°C. This met the criteria of the second hypothesis established in AIM 1 in that adaptation would look different at the two temperatures.

In addition, this further supports the findings from removing trial 1 from analysis – without an initial stimulus that causes a great change in hemodynamic response, minimal to no adaptation is observed as there is nothing to adapt to.

2.5 Conclusion

In conclusion, the goal of this investigation was met as we observed adaptation through measurements from the fNIRS signal, by monitoring the effect of repeated exposure to experimental pain on hemodynamics in the prefrontal cortex region. The hemodynamic response to repeated exposure of noxious stimuli (4°C) shows a diminished response over time when monitored by fNIRS. Innocuous stimuli (15°C) did not show significant adaptation, which can be explained in that the initial exposure did not cause a great change to the system, and thus, did not warran adaptation. Upon further study, this information about adaptation and the way subjects respond to repeated exposure, can show clinicians the state of an individuals' pain pathways, allowing them to apply treatment sooner rather than later, and in a more targeted way.

2.6 Acknowledgement

We would like to thank Dr. Zeinab Barati for her advice and support in advancing the use of functional near infrared spectroscopy as a diagnostic tool for pain assessment – continuing to take advantage of the robust and versatile nature of fNIRS.

Chapter 3

SPECIFIC AIM 2. Perception of Pain and Adaptation. In this aim, we want to see if there is a correlation between the perceived adaptation to pain and the fNIRS signal.

3.1 Introduction

The perception of pain is a complicated phenomenon as it combines dynamic interplay between the peripheral and central nervous system. Due to the unpleasantness of pain, it is something that is avoided, yet necessary, which adds to its' complexity. Its necessity stems from the awareness it gives of any external danger in the environment that may be harmful to the body. Even further, acute pain is adaptive and results in survival actions being taken such as escaping behaviors. In contrast, chronic pain is maladaptive causing suffering and a great reduction in life expectancy. The issue with pain in general is that the human pain conditions present in a multitude of biological and psychological phenotypes, in particular, idiopathic pain disorders. Environmental events that invoke tissue injury and psychological distress also add to the difficulty for diagnosis due to the way that they can influence amplification of pain. Realizing the importance of this as well as understanding that pain is a personal experience where generalizations are not always applicable, this investigation proposes the pairing of subjective pain measures with the objective hemodynamic response to pain in an effort to see the correlation between these two.

While these past studies have compared fNIRS signals to subjects' pain reporting using the visual analog scale (VAS) or the numerical pain rating scale (NRS-11), further analysis beyond this is necessary to continue to improve the utility of fNIRS in quantifying pain. This will be done by assessing the sensitivity of fNIRS in quantifying pain experiences as they relate to the individual. (Barati et al., 2013)

In the first aim, we saw that when looking at ΔHBO2 and ΔHB, we observed a diminished response after repeated exposure to noxious stimuli (adaptation over time). The next step to answer the question of how the perception of adaptation compares to the hemodynamic response of adaptation observed through fNIRS. As a result, in AIM 2 we intend to expand on the previous fNIRS studies relating to adaptation, by including more psychological measures such as the McGill Pain Questionnaire and the State Trait Anxiety Inventory. From this, we want to look at how the fNIRS signal looks when compared to a subjects' perception. Pain scores (pain level from 0-10) have proven to be a good tool in predicting a subjects' response to pain. With this, we will perform correlation analyses to see how strongly (or weakly) correlated the measures of perception are when looking at the adaptation trend in the fNIRS data.

3.2 Materials and Methods

Experimental Approach (n=16): Two subjective questionnaire were used as a means to asses each individuals pain experience, the State Trait Anxiety Inventory (STAI) and the Short-Form McGill Pain Questionnaire (SF-MPQ). The STAI gives a measure of two anxiety constructs, state and trait, the former being a measure of the tendency to anxiously in response to a stimulus that is perceived as threatening and the latter being a measure of arousal in response to a stimuli that is seen as dangerous (R Melzack, 1987). This inventory was given before data collection began and after it was complete. This would assist in distinguishing a subjects' feeling of anxiety upon entering the study, and anxiety that was caused by the study. As pain and anxiety are linked through the stress that is accompanied through painful stimulation experiences, this measure proved to be useful during analysis. (R Melzack, 2001)

The MPQ has become a standard questionnaire used to quantify the subjective pain experience (R Melzack, 1987). As this questionnaire is 40 questions in length, for timing purposes

during experimentation, the SF-MPQ was used. Both forms serve the same purpose in that they measure subjective pain through analyzing sensory and affective pain descriptors, the former focusing on the emotional and feeling response to pain and the latter focusing on the quality, intensity and location of pain. This questionnaire was asked after the first-hand immersion in the 4°C water bath, after the mid-point hand immersion in the 4°C water bath and lastly after the final hand immersion in the 4°C water bath. This tool will give information as to whether the pain experienced by the individual changes throughout the experiment.

Following the experimental model used in Barati et al., two fNIRS probes of the same configuration were secured on each subjects' forehead at a position proximal to the anterior median line.

After each CPT when subjects were asked to place their hand in tepid water, in addition to the questionnaires that were asked at the time described above, subjects were asked to rate their pain on a numerical scale of 0 to 10 (NIRS-11). The scale defining '0' as no pain and '10' being the worst pain imaginable. (R Melzack, 1987; Sjolund & Persson, 2007)

Statistical Analysis

To compare the fNIRS data with the subjective pain measures, the approach used here is that of simple linear regression. This type of regression calculates the equation that minimizes the distance between the fitted line and all the data points. On each axis are the two measures that are to be correlated. The linear correlation coefficient (r) looks at both the strength and direction of the linear relationship between the two variables of interest. The coefficient of determination (r^2) gives the amount of predictable variance between the two variables. (Ravn, Frederiksen, Skovsen, Christrup, & Werner, 2012; Tak & Ye, 2014)

3.2.1 Subjects

Healthy subjects (n=16) with no history of neurological, psychiatric or psychological disorders and who were not currently on any medications were recruited from the Drexel University community. The Edinburgh Handedness Inventory, confirmed that subjects were right handed before study participation was allowed. When subjects clearly confirmed their understanding of the experimental procedures that they are voluntarily participating in, they were required to sign the informed consent document before participating in any further study related activities. The Drexel IRB, through the university's Human Research Protection Program, approved all study proceedings.

3.2.2 Protocol

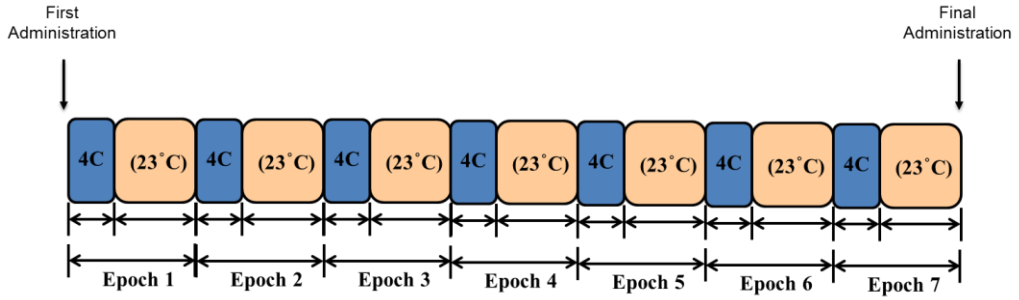


Figure 28. State trait anxiety inventory administration protocol.

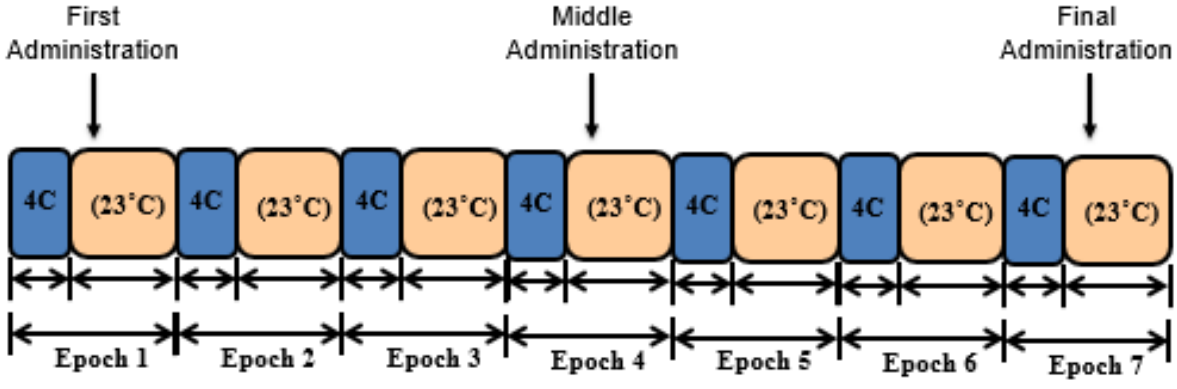


Figure 29. Short-Form McGill Pain Questionnaire administration protocol.

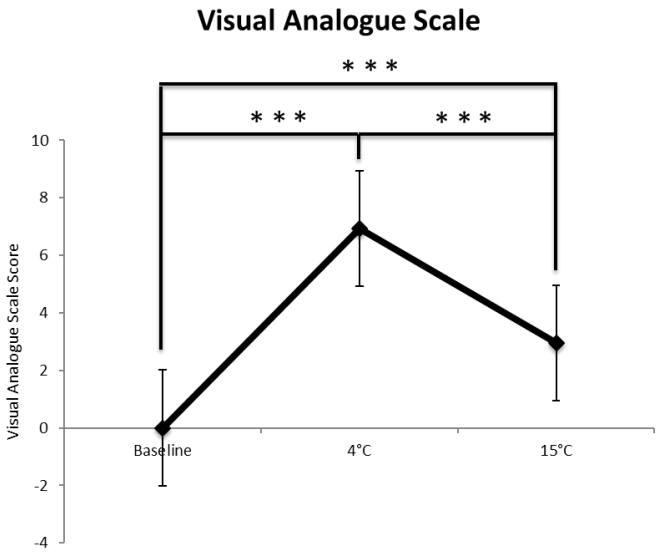
3.2.3 Measurements

See section 2.3.1 and 2.3.4 for details on fNIRS measurements and analysis.

3.3 Results

3.3.1 fNIRS Signal Correlation with the Visual Analogue Scale at 4 degrees Celsius and 15 degrees Celsius

VAS demonstrates a significant difference between pain experienced at 4°C and 15°C (Baseline mean VAS=0 +/- 0; 4˚C mean VAS=6.93 +/- 0.4137; 15˚C mean VAS=2.943 +/- 1.9221). Mean displayed with SE bars (n=16). This was identified through the use of a two-tailed t-test for comparison. Baseline vs. 4˚C: p=0.003; Baseline vs. 15˚C: p=0.003; 4°C vs. 15°C: p=0.001. A graphical display of these results can be found in Figure 30. Significance of change is identified in this chapter by the following designations: *=Significance of p< 0.10, **=Significance of p <0.05, and ***=Significance of p < 0.01. What this shows is that between baseline and 4°C, baseline and 15°C, and 4°C and 15°C, there was a significant difference between the average pain score over all trials for a given temperature (excluding baseline as this was a measure of pain score without any stimuli given).



VAS demonstrates a significant difference between pain experienced at 4°C and 15°C (Baseline mean VAS=0 +/- 0; 4˚C mean VAS=6.93 +/- 0.4137; 15˚C mean VAS=2.943 +/- 1.9221; p=0.000). Mean displayed with SE bars (n=15). Baseline vs. 15C: p=0.000; Baseline vs. 15C: p=0.000; 4C vs. 15C: p=0.000

3.3.2 fNIRS Signal Correlation (Deoxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C

The scatter plot when using channels 3, 5 and 6, which were the channels that demonstrated significance for ΔHB are shown in Figure 31. Although the linear regression results show a slightly positive correlation (the greater the change in deoxyhemoglobin, the higher the pain score), the results based on the r (r=0.286) and R^2 (R^2 = 0.082) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 228.269).

The linear regression results when using only channel 5, which was the most significant for ΔHB, show a slightly positive correlation (the greater the change in deoxyhemoglobin, the higher the pain score). The results based on the r (r=0.0.091) and R^2 (R^2 = 0.008) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 246.532).

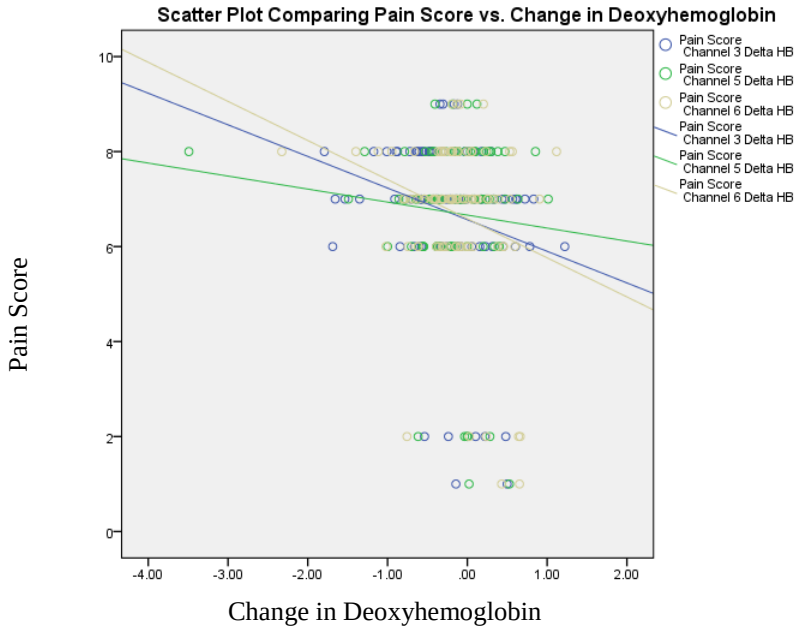


Figure 31. Scatter plot with changes in deoxyhemoglobin (channels 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.3 fNIRS Signal Correlation (Oxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C

The scatter plot when using only channels 2, 3, 5 and 6 which were the most significant for ΔHBO_2 are shown in Figure 32. Although the linear regression results show a slightly positive correlation (the greater the change in oxyhemoglobin, the higher the pain score), the results based on the r (r=0.374) and R^2 (R^2 = 0.140) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 213.888).

The linear regression results when using only channels 5, which was the most significant for ΔHBO_2, show a slightly positive slope (the greater the change in oxyhemoglobin, the higher the pain score). The results based on the r (r=0.127) and R^2 (R^2 = 0.016) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 244.587).

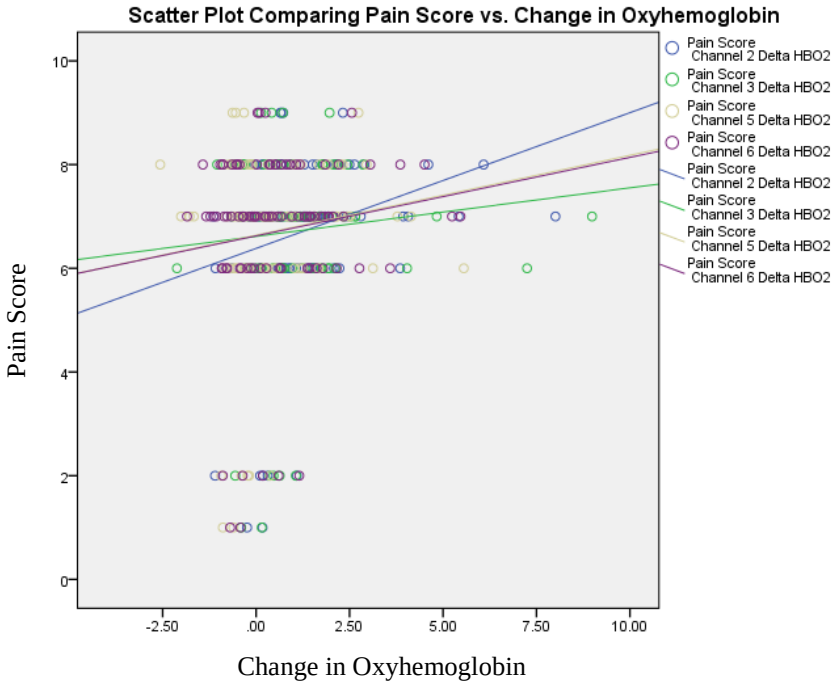


Figure 32. Scatter plot with changes in oxyhemoglobin (channels 2, 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.4 fNIRS Signal Correlation (Deoxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C with only Trial 1 Data

From AIM 1, it was identified that trial 1 presents significantly different data from trials 2 through 6. As a result, there are 8 additional linear regressions that were performed here. The next four will place a focus on trial 1, with the subsequent for focusing on trials 2 through 6.

The scatter plot when using channels 3, 5 and 6, which were the channels that demonstrated significance for ΔHB (while only focusing on trial 1) are shown in Figure 33. Although the linear regression results show a slightly positive correlation, the results based on the r ($r=0.427$) and R^2 ($R^2 = 0.182$) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 25.287).

The linear regression results when using only channel 5, which was the most significant for ΔHB (while focusing only on trial 1) show a slightly positive correlation. The results based on the r ($r=0.106$) and R^2 ($R^2 = 0.011$) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 30.575).

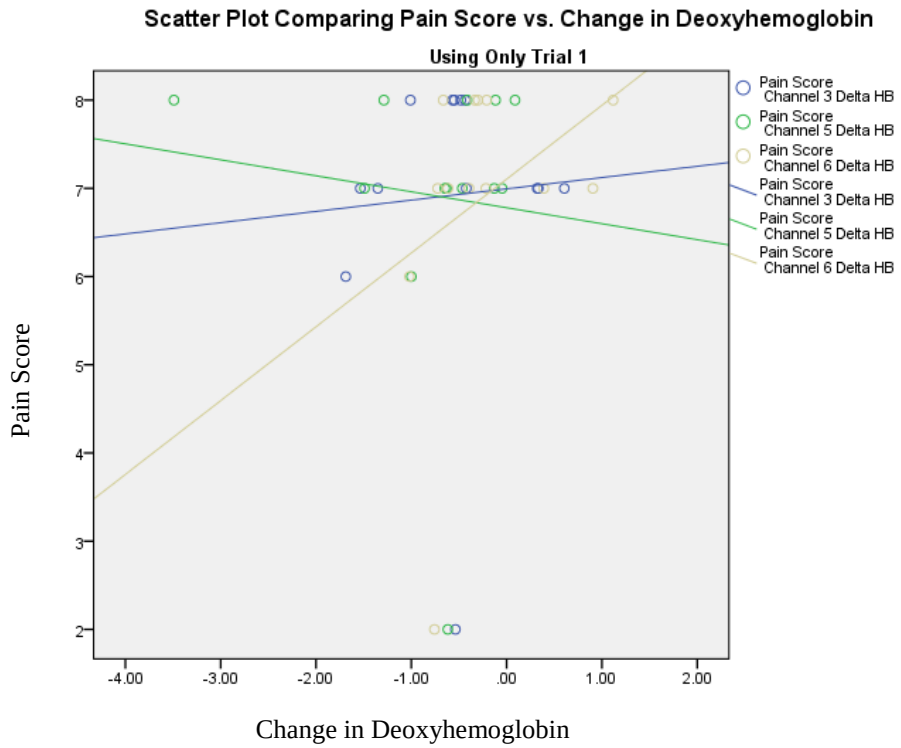


Figure 33. Simple linear regression plot with changes in deoxyhemoglobin (channels 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.5 fNIRS Signal Correlation (Oxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C with only Trial 1 Data

The scatter plot when using only channels 2, 3, 5 and 6 which were the most significant for ΔHBO_2 (while focusing only on trial 1) are shown in Figure 34. Although the linear regression results show a slightly positive correlation, the results based on the r (r=0.420) and R^2 (R^2 = 0.176) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 25.473).

The linear regression results when using only channels 5 which was the most significant for ΔHBO_2, show a slightly positive correlation. The results based on the r (r=0.028) and R^2 (R^2 = 0.001) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 30.898).

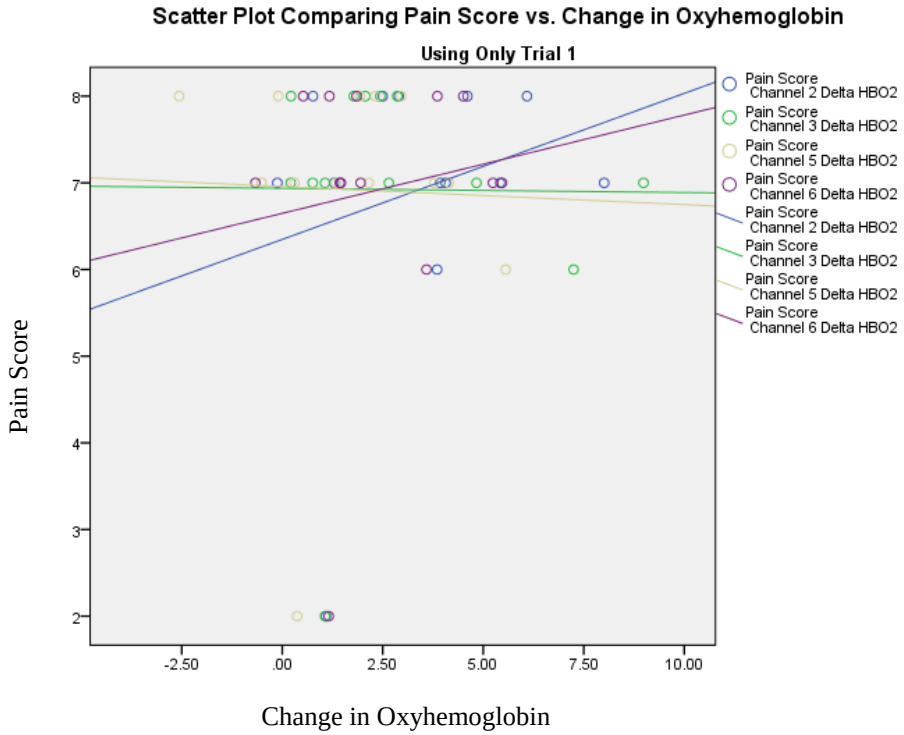


Figure 34. Scatter plot with changes in oxyhemoglobin (channels 2, 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.6 fNIRS Signal Correlation (Deoxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C after Removing Trial 1 Data

Upon completing the linear regression analysis for these 4 analyses, we continued the investigation by repeating the same 4 analyses after removing trial 1 from the data. As we observed in AIM 1, trial 1 gives a response indicative of initial exposure to a stimulus, and is not representative of the adaptation that follows. To corroborate these findings, we proceeded with the analyses seen below.

The scatter plot when using channels 3, 5 and 6, which were the channels that demonstrated significance for ΔHB (after removing trial 1 signals from the data) are shown in Figure 35. Although the linear regression results show a slightly positive correlation (the greater the change in deoxyhemoglobin, the higher the pain score), the results based on the r (r=0.417) and R^2 (R^2 = 0.174) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 179.183).

The linear regression results when using channel 5, which was the channel that demonstrated significance for ΔHB (after removing trial 1 signals from the data), show a slightly positive correlation (the greater the change in deoxyhemoglobin, the higher the pain score). The results based on the r (r=0.074) and R^2 (R^2 = 0.005) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 215.987).

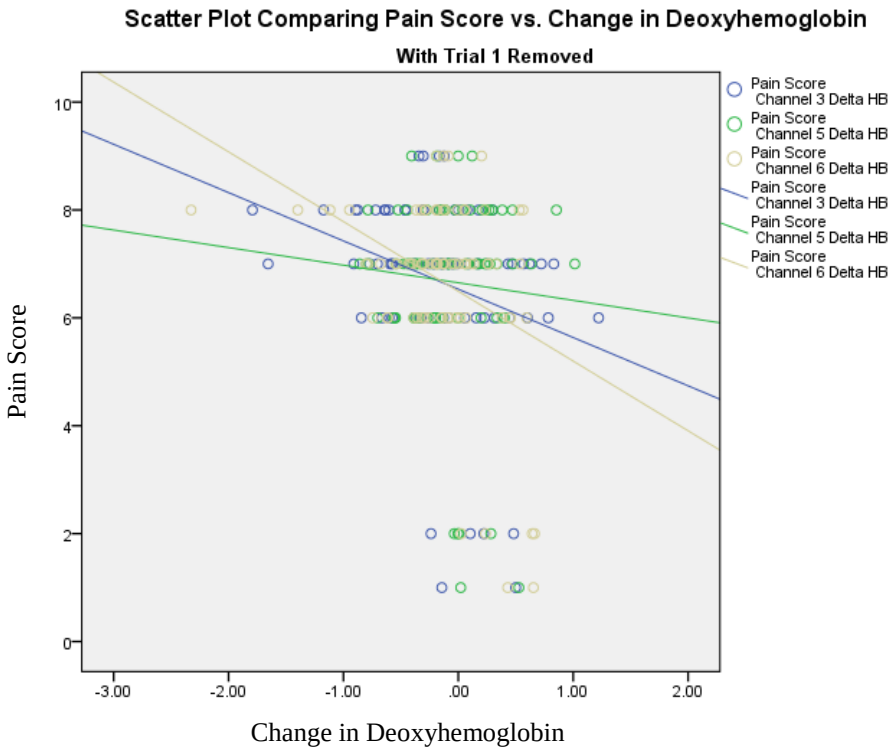


Figure 35. Scatter plot with changes in deoxyhemoglobin (channels 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.7 fNIRS Signal Correlation (Oxyhemoglobin) with the Visual Analogue Scale Pain Scores using Simple Linear Regression at 4°C after Removing Trial 1 Data

The scatter plot when using only channels 2, 3, 5 and 6 which were the most significant for ΔHBO_2 (after removing trial 1 signals from the data) are shown in Figure 36. Although the linear regression results show a slightly positive correlation (the greater the change in oxyhemoglobin, the higher the pain score), the results based on the r (r=0.437) and R^2 (R^2 = 0.191) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 175.456).

The linear regression results when using only channels 5, which was the most significant for ΔHBO_2 (after removing trial 1 signals from the data), show a slightly positive correlation (the greater the change in oxyhemoglobin, the higher the pain score). The results based on the r (r=0.168) and R^2 (R^2 = 0.028) value show that there is no definitive correlation between fNIRS signal and subjects' pain score (residual sum of squares = 210.888).

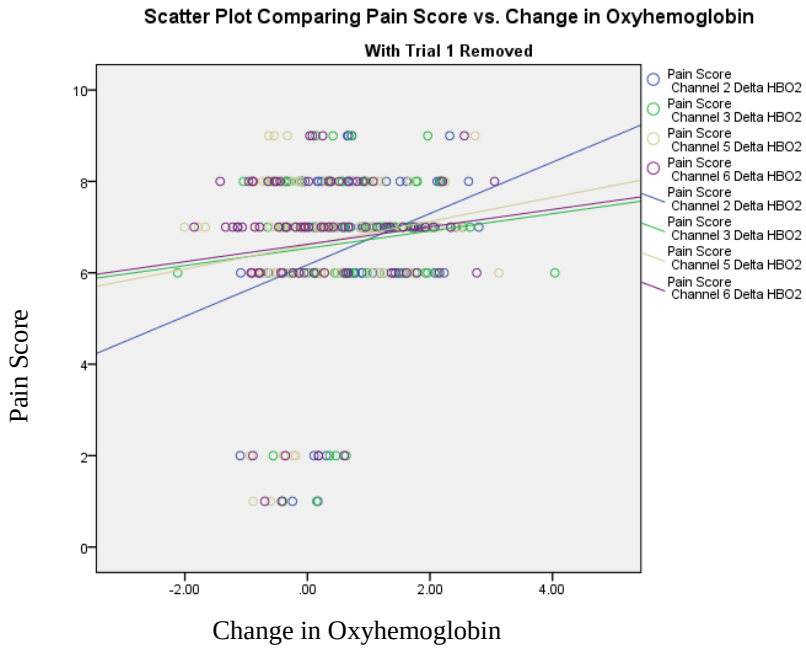


Figure 36. Scatter plot with changes in oxyhemoglobin (channels 2, 3, 5 and 6) on the x-axis and pain score on the y-axis.

3.3.8 State Trait Anxiety Inventory Results

Effect of Stimuli via the CPT through State Anxiety Inventory score (STAI-s) State-Trait Anxiety Inventory Analysis (n=16) Mean displayed with standard error (SE) bars (Baseline mean STAI-s=26.79 +/- 1.71; Post-4˚C mean STAI-s=35.57 +/- 3.17; Post-15°C mean STAI-s=32.54 +/- 2.40. This was identified using a two-tailed t-test for comparison. Baseline vs. 4°C: p=0.011; Baseline vs. 15°C: p=0.036; Post-4˚C mean vs. Post-15°C: p=0.5016. These results are graphically displayed in Figure 37.

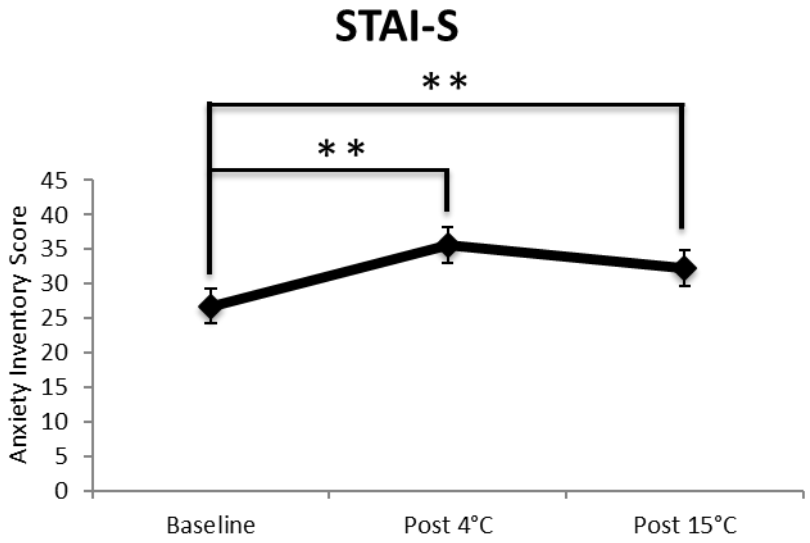


Figure 37. State trait anxiety inventory – state anxiety score comparison between baseline, 4 degrees Celsius and 15 degrees Celsius.

3.3.9 Short Form – McGill Pain Questionnaire Results at 4 degrees Celsius

For the case of sensory pain scores at 4 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C (Baseline mean Sensory pain score=0 +/- 0; 4˚C Trial 1 mean sensory pain score=16.85 +/- 1.75; 4˚C Trial 7 sensory pain score=16.85 +/- 2.01; df = 15). These results are seen in Figure 38.

This was identified by using a two-tailed t-test for comparison. Mean displayed with SE bars (n=16). Baseline vs. 4˚C Trial 1: p=0.010; Baseline vs. 4˚C Trial 7: p=0.008; 4˚C Trial 1 vs. 4˚C Trial 7: p=1.000.

For the case of affective pain scores at 4 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C (Baseline mean Affective pain score=0 +/- 0; 4˚C Trial 1 mean affective pain score=4.54 +/- 0.75; 4˚C Trial 7 affective pain score=4.31 +/- 0.74; df = 15). These results are highlighted in Figure 39. This was identified through using a two-tailed t-test for comparison. Mean displayed with SE bars (n=16). Baseline vs. 4˚C Trial 1: p=0.006; Baseline vs. 4˚C Trial 7: p=0.009; 4˚C Trial 1 vs. 4˚C Trial 7: p=0.489.

For the case of total McGill Pain Questionnaire scores at 4 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C (Baseline mean total SF-MPQ pain score=0 +/- 0; 4˚C Trial 1 mean total SF-MPQ pain score=21.39 +/- 1.96; 4˚C Trial 7 mean total SF-MPQ pain score=19.643 +/- 2.723; df = 15). These results are displayed in Figure 40. This was identified through using a two-tailed t-test for comparison. Mean displayed with SE bars (n=16). Baseline vs. 4˚C Trial 1: p=0.002; Baseline vs. 4˚C Trial 7: p=0.006; 4˚C Trial 1 vs. 4˚C Trial 7: p=0.435.

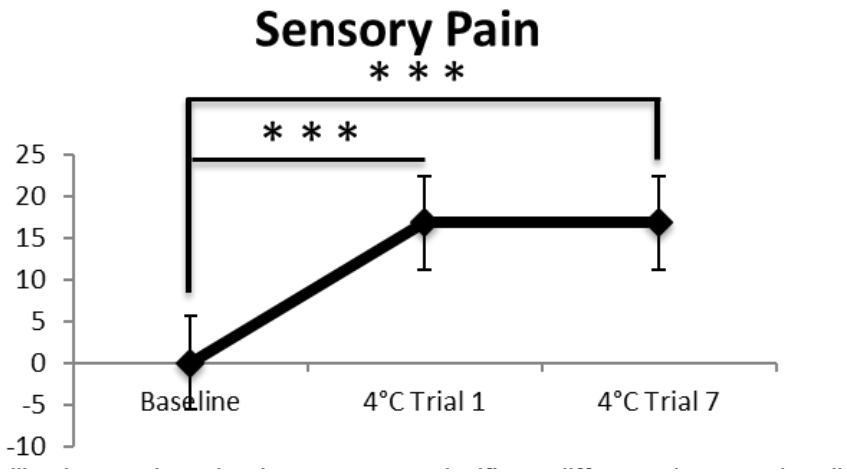


Figure 38. Short-Form McGill Pain Questionnaire – sensory pain score comparison between baseline, 4 degrees Celsius Trial 1 and 4 degrees Celsius Trial 7.

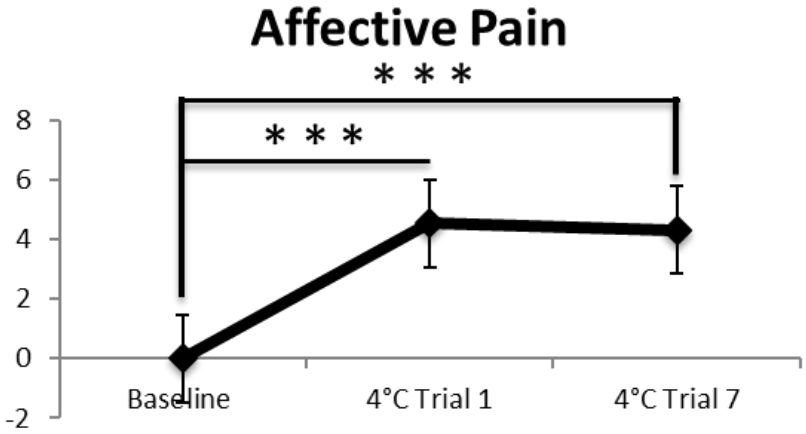


Figure 39. Short-Form McGill Pain Questionnaire – affective pain score comparison between baseline, 4 degrees Celsius Trial 1 and 4 degrees Celsius Trial 7.

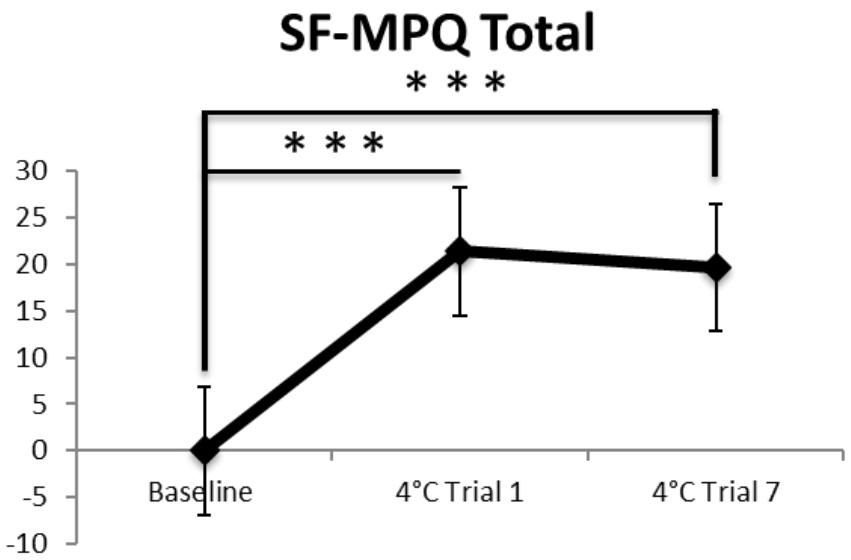


Figure 40. Short-Form McGill Pain Questionnaire – total score comparison between baseline, 4 degrees Celsius Trial 1 and 4 degrees Celsius Trial 7.

3.3.10 Short Form – McGill Pain Questionnaire Results at 15 degrees Celsius

For the case of sensory pain scores at 15 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 15°C (Baseline mean Sensory pain score=0 +/- 0; 15˚C Trial 1 mean sensory pain score=8.11 +/- 2.08; 15˚C Trial 7 sensory pain score=8.33 +/- 3.33; df = 15). Mean displayed with SE bars (n=16). This is seen in Figure 41. This was identified by a two-tailed t-test for comparison. Baseline vs. 15˚C Trial 1: p=0.004; Baseline vs. 15˚C Trial 7: p=0.037; 15˚C Trial 1 vs. 15˚C Trial 7: p=0.901.

For the case of the affective pain scores at 15 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 15°C (Baseline mean Affective pain score=0 +/- 0; 15˚C Trial 1 mean affective pain score=2.22 +/- 0.98; 15˚C Trial 7 affective pain score=3.11 +/- 1.37; df = 15). Mean displayed

with SE bars (n=16). This is seen in figure 42. This was identified with a two-tailed t-test for comparison. Baseline vs. 15˚C Trial 1: p=0.054; Baseline vs. 15˚C Trial 7: p=0.053; 15˚C Trial 1 vs. 15˚C Trial 7: p=0.069.

For the case of total McGill Pain Questionnaire scores at 4 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C (Baseline mean total SF-MPQ pain score=0 +/- 0; 15˚C Trial 1 mean total SF-MPQ pain score=9.30 +/- 2.85; 15˚C Trial 7 mean total SF-MPQ pain score=10.30 +/- 4.35; df = 15). Mean displayed with SE bars (n=16). This is seen in Figure 43. This was identified through using a two-tailed t-test for comparison. Baseline vs. 15˚C Trial 1: p=0.010; Baseline vs. 15˚C Trial 7: p=0.042; 15˚C Trial 1 vs. 15˚C Trial 7: p=0.603.

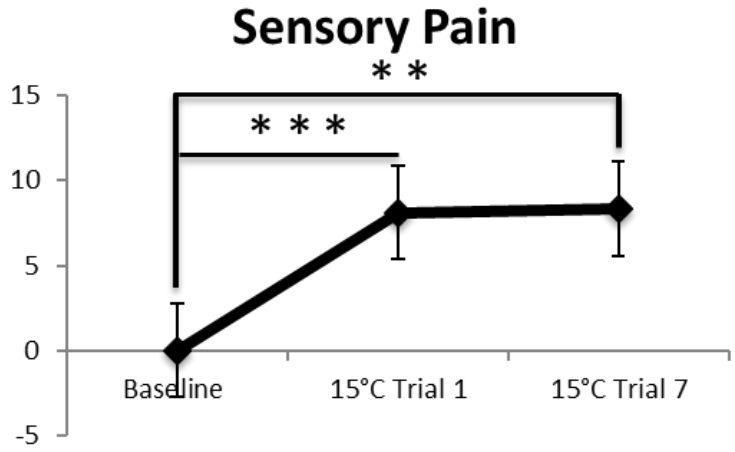


Figure 41. Short-Form McGill Pain Questionnaire – sensory pain score comparison between baseline, 15 degrees Celsius Trial 1 and 15 degrees Celsius Trial 7.

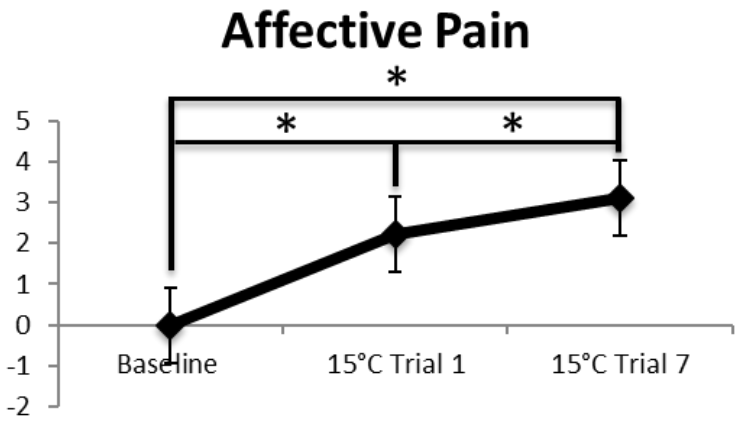


Figure 42. Short-Form McGill Pain Questionnaire – affective pain score comparison between baseline, 15 degrees Celsius Trial 1 and 15 degrees Celsius Trial 7.

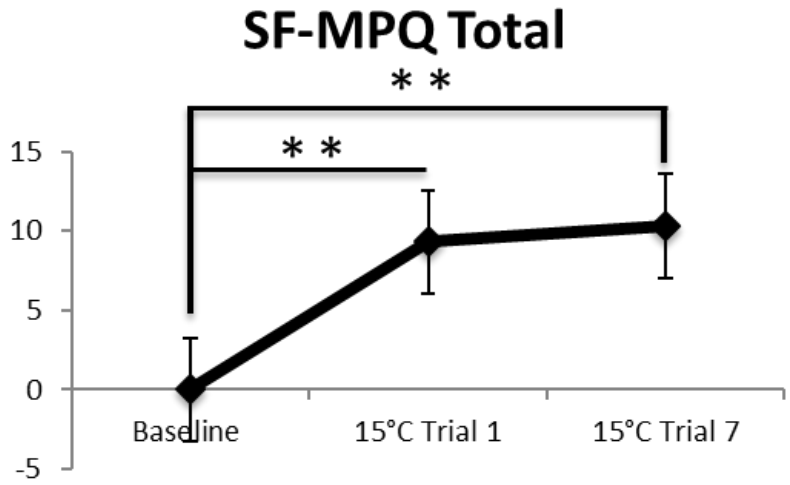


Figure 43. Short-Form McGill Pain Questionnaire – total score comparison between baseline, 15 degrees Celsius Trial 1 and 15 degrees Celsius Trial 7.

3.3.11 Short Form – McGill Pain Questionnaire Results Comparing 4 degrees Celsius and 15 degrees Celsius

For the case of sensory pain scores when comparing 4 degrees Celsius to 15 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C and 15°C respectively, and pain experienced at 4°C and 15°C (Baseline mean Sensory pain score=0 +/- 0; 4˚C Trial 1 mean sensory pain score=16.85 +/- 1.75; 15˚C Trial 1 mean sensory pain score=8.11 +/- 2.08; d = 15). Mean displayed with SE bars (n=16). This is seen in Figure 44. This was identified through the use of a two-tailed t-test for comparison. Baseline vs. 4˚C Trial 1: p=0.001; Baseline vs. 15˚C Trial 1: p=0.001; 4˚C Trial 1 vs. 15˚C Trial 1: p=0.001.

For the case of the affective pain scores when comparing 4 degrees Celsius to 15 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C and 15°C respectively, and pain experienced at 4°C and 15°C (Baseline mean Affective pain score=0 +/- 0; 4˚C Trial 1 mean affective pain score=4.54 +/- 0.74; 15˚C Trial 1 mean affective pain score=2.22 +/- 0.98; df = 15). Mean displayed with SE bars (n=16). This is seen in Figure 45. This was identified through the use of a two-tailed t-test for comparison. Baseline vs. 4˚C Trial 1: p=0.001; Baseline vs. 15˚C Trial 1: p=0.006; 4˚C Trial 1 vs. 15˚C Trial 1: p=0.025.

For the case of total McGill Pain Questionnaire scores when comparing 4 degrees Celsius and 15 degrees Celsius, the McGill pain questionnaire demonstrates a significant difference between baseline pain experience and pain experienced at 4°C and 15°C respectively, and pain experienced at 4°C and 15°C (Baseline mean total SF-MPQ pain score=0 +/- 0; 4˚C Trial 1 mean total SF-MPQ pain score=21.39 +/- 1.96; 15˚C Trial 1 mean total SF-MPQ pain score=9.30 +/-

2.163; df = 15). Mean displayed with SE bars (n=16). This is seen in Figure 46. This was identified through the use of a two-tailed t-test for comparison. Baseline vs. 4˚C Trial 1: p=2.100E-4; Baseline vs. 15˚C Trial 1: p=0.001; 4˚C Trial 1 vs. 15˚C Trial 1: p=0.003.

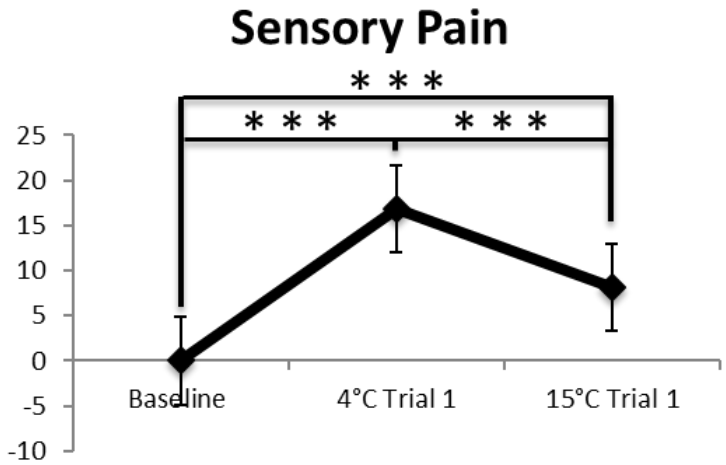


Figure 44. Short-Form McGill Pain Questionnaire – sensory pain score comparison between baseline, 4 degrees Celsius Trial 1 and 15 degrees Celsius Trial 1.

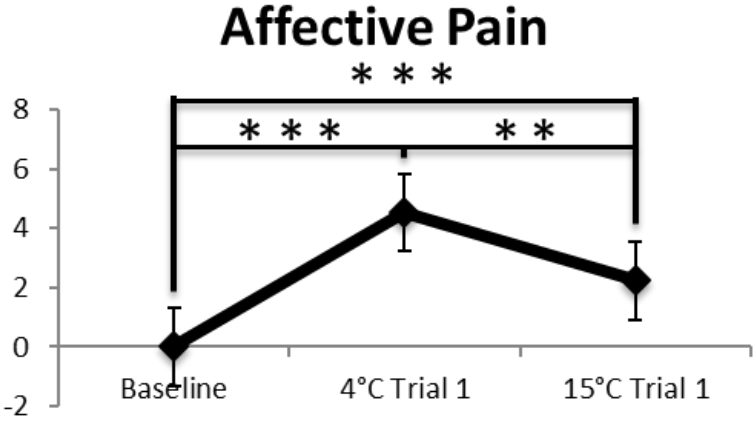


Figure 45. Short-Form McGill Pain Questionnaire – affective pain score comparison between baseline, 4 degrees Celsius Trial 1 and 15 degrees Celsius Trial 1.

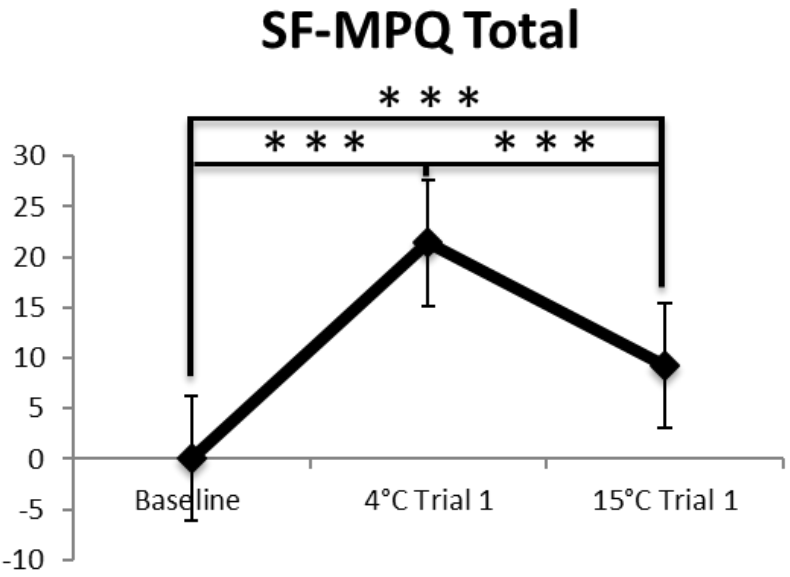


Figure 46. Short-Form McGill Pain Questionnaire – total score comparison between baseline, 4 degrees Celsius Trial 1 and 15 degrees Celsius Trial 1.

3.3.12 Results Overview

First, we looked at the visual analogue scale (VAS) pain score to see how perception of adaptation was experienced before comparing it with the fNIRS signal. The hypothesis here was that there would be a correlation between perception and the hemodynamic response. What was seen is that the results were significantly different from baseline to 4°C and from 4°C to 15°C respectively. There was also a significant difference between baseline pain scores and 15°C pain scores. Results not shown are VAS scores from the first trial of stimulus exposure to the last trial. These results did not differ significantly alone from trial to trial. This was not expected as in many previous studies, including studies conducted in our lab (Barati et al., 2013), when comparing noxious temperature perception, and innocuous temperature perception, there was a clear correlation between the fNIRS signal and perception. However, when conducting the experiment in this investigation in a repeated measures fashion, the results do not differ from trial to trial significantly – proving that pain perception continues to be subjective, personalized, and easily influenced. (Keogh & Herdenfeldt, 2002; Loeser & Melzack, 1999; Salomons & Johnstone, 2007; Soetanto J.; Wong, T., 2006)

The simple linear regression results presented here all show that while there may be a correlation, that it is weak in nature and cannot definitively suggest a strong correlation with the fNIRS signals that are representative of all trials when compared to pain scores. In looking only at trial 1 data and performing the same correlation analysis, what we see is that although the pain scores are rather high, this does not correlate with any level of change in oxy and deoxy hemoglobin, suggesting that more subjects may need to be collected. Further, when removing trial 1 from the analysis, the results showed the same as when trial 1 was included (no strong correlation); however, the correlation was even weaker, agreeing with the results of AIM 1 in that

trial 1 is necessary to initiate a bodily adaptation, because if there is not a significant external stimulus, then no adaptation is likely to show (Taylor & Taylor, 1956). This bolsters findings previously identified in the literature that adaptation happens within the first few trials of repeated exposure experimentation when processing sensory information. (Hollins, Harper, & Maixner, 2011)

Regarding STAI anxiety scores, there was a significant difference between baseline and 4 degrees Celsius as well as baseline and 15 degrees Celsius.

When looking at sensory pain scores and total pain scores for both 4 degrees Celsius and 15 degrees Celsius, there was a significant difference between the baseline sensory pain and the sensory pain score after trial 1, as well as a significant difference between the baseline sensory pain and the sensory pain score after trial 7. These results were the same for the total McGill Pain Questionnaire scores.

In regards to affective pain, at 4 degrees Celsius there was a significant difference between the baseline affective pain and the affective pain score after trial 1, as well as a significant difference between the baseline affective pain and the affective pain score after trial 7. This was the same at 15 degrees Celsius; however, there was also a significant difference between the affective pain score after trial 1 and after trial 7.

When comparing baseline McGill Pain Questionnaire scores to 4 degrees Celsius trial 1 scores and 15 degrees Celsius trial 1 scores respectively, there was a significant difference in both cases regarding sensory, affective, and total scores. This was also the case when comparing 4 degrees Celsius trial 1 scores to 15 degrees Celsius trial 1 scores.

3.4 Discussion

Only significant channels were carried over to this part of the analysis because the non-significant channels did not show adaptation as defined by fNIRS (described in the discussion of Chapter 2). Including the non-significant channels may skew the data, deviating from the focus on adaptation, so only significant channels were assessed. In addition to this, trial 1 was included in all analyses performed here because based on the data from AIM 1, to observe adaptation, the initial exposure needs to cause a great enough change to the system that would cause a need to adjust back to stability.

The information from the simple linear regression when comparing the significant channels from AIM 1 to the measures of perception identified in AIM 2, is informative in that while the autonomic response may decrease over time (adaptation), the pain score does not change in the same way. The data from conducting the analysis with these specifications suggests that while the body physiologically adapts, the perception of the pain (being more complex (Donald, 2000)) may not react in the same way (Bodnar, Kelly, Spiaggia, & Glusman, 2016). Further, additional subjects may be needed to increase the significance of the results shown before being able to definitively conclude how fNIRS compares with measures of perception.

When looking at measures of perception alone, when comparing the two temperatures of 4°C and 15°C, there is a great difference in perception; however, repeated exposure to the same temperature does not cause a change in perception that correlates with the decrease in autonomic response. Thus, the hypothesis tested failed when held up to the results shown in the data. Even though the results did not demonstrate a significant correlation, based on the trend that appears from the data, studying more subjects may strengthen the findings obtained and drive down the error, as well as increase the significance behind the results.

3.5 Conclusion

The goal of the analysis conducted in this chapter was achieved in that we obtained results that allowed us to look at the relationship between the perceived adaptation to pain (perception measures) and the fNIRS signal. The results highlighted in this chapter advanced the progress of the overall goal of investigating the effect of the repeated exposure experimentation on the hemodynamic response, because from this evaluation, when comparing the fNIRS signal to perception, they do not change in the same way with regard to repeated exposure experimentation. This informs us that the bodies' response may be more objective in nature than the response based on the awareness of an individual to their pain experience. (Bodnar et al., 2016; Donald, 2000)

3.6 Acknowledgement

Authors acknowledge Dr. Robert Melzack for his great contributions in the pioneering the initiation of the focus on pain perception.

Chapter 4

SPECIFIC AIM 3. Exploratory Analysis of fNIRS Clustering in Adaptation

4.1 Introduction

In machine learning, there are two groups that techniques typically fall under, supervised or unsupervised. In supervised machine learning, the output is given for datasets for the creation of the algorithm upon training. In unsupervised learning, there is no output given, and the goal is simply to be able to group the data based on the data itself. The goal typically in unsupervised learning is to group together items that are "close" in a multidimensional feature space (Brock, Pihur, Datta, & Datta, 2008). This gives us insight into the data itself and the separations that can be achieved. One of the most common clustering methods used is hierarchical clustering where objects are grouped together based on distance from one another. The complete linkage method was used to cluster the data here in that clusters are sequentially built on top of one another into larger clusters; ultimately resulting in a dendrogram with results of the hierarchy achieved in the clustering of the data set at hand. (Brock et al., 2008)

What this would look like in the case of this study is being able to separate the hemodynamic response of specific trials into specific groups. As adaptation should have occurred over repeated exposure to stimuli, the response should differ from trial to trial. In the scope of this aim, the goal was to cluster the data based on trial number, following the hypothesis that there is a significant difference in subjects' response based on trial to the point of easy separation through complete-linkage clustering.

4.2 Materials and Methods

4.2.1 Subjects

See section 2.3.2 and section 3.3.1 for details on the subject recruitment methods as these subjects were recruited in the same way as those in Chapter 2 and Chapter 3.

4.2.3 Protocol

See section 2.3.3 for details on protocol used as this experimental protocol followed the same steps as those outlined in Chapter 2.

4.2.3 Measurements

See section 2.3.1 and 2.3.4 for details on fNIRS measurements and analysis.

Statistical Analysis

To perform the clustering analysis, the selected method was average-linkage clustering. Average linkage clustering can be defined as the average distance between each point in one cluster to points in other clusters and has recently been used by our lab to automate the process of fNIRS signal separation into meaningful categories (Pourshoghi, Zakeri, & Pourrezaei, 2016). This type of clustering is standard to use to visualize the data. Similar to the analysis of AIM 2, the channels used in this analysis are only those that demonstrated significance in AIM 1. This would provide the best data for optimal clustering. In addition to average hierarchical clustering, k-means clustering was also used in this exploratory analysis. This divides clusters into groups where each data point congregates to a cluster with the nearest mean. This ultimately yields to groups of data points that are close in value (or have the same mean).

4.3 Results

4.3.1 fNIRS Signal Cluster Dendrograms

Average linkage hierarchical clustering performed for the fNIRS signals from subjects who underwent the experimental process in AIM 1 for 4°C, divided into 4 distinct clusters when using average linkage clustering. Figure 47 visually shows the clusters, and table 5 shows a matrix of accuracies based on trials. The channels of significance for oxyhemoglobin (channels 2, 3, 5 and 6) and deoxyhemoglobin (channels 3, 5 and 6) were used for this analysis. What this shows is how well the clustering algorithm could differentiate between different trials. From the data in Table 5, while trials 3, 4 and 5 seem to gather in cluster 2 with a higher percentage, there is no apparent dominance of any trial in any cluster.

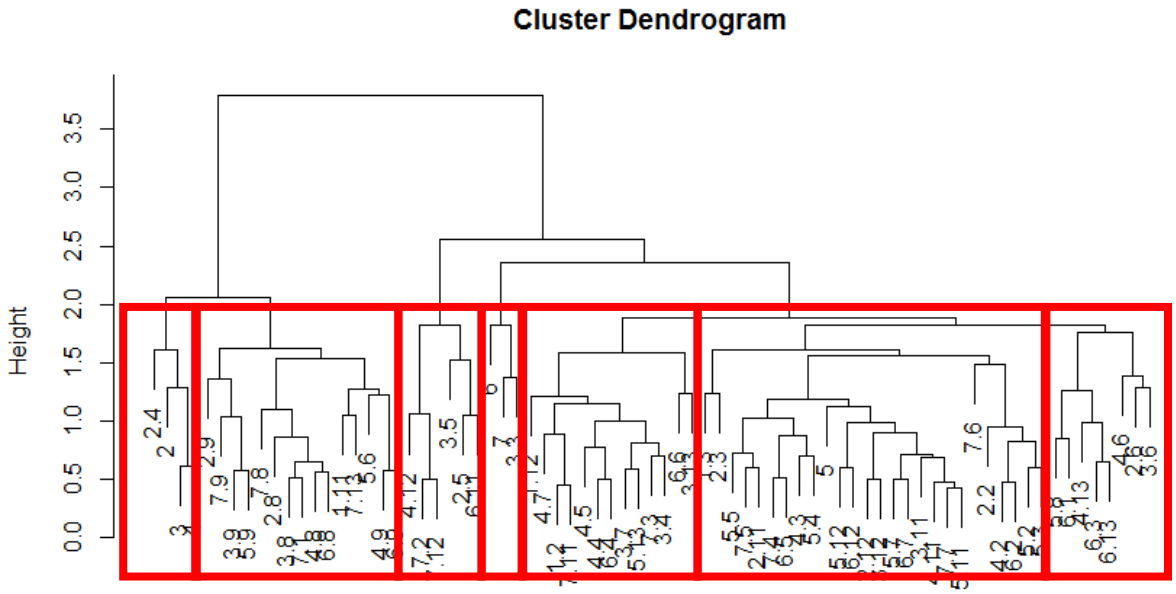


Figure 47. Average clustering analysis using channels 3, 5 and 6 from the change in deoxyhemoglobin and channels 2, 3, 5 and 6 from the change in oxyhemoglobin; cluster 1 begins on the left and the subsequent trials follow sequentially ending with cluster 4 being the rightmost cluster.

	Cluster 1	Cluster2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Trial 1	0.00%	25.00%	0.00%	0.00%	50.00%	25.00%	0.00
Trial 2	20.00%	20.00%	10.00%	0.00%	0.00%	40.00%	10.00
Trial 3	9.09%	18.18%	9.09%	9.09%	27.27%	18.18%	9.09
Trial 4	8.33%	16.67%	8.33%	0.00%	25.00	25.005	16.67
Trial 5	0.00%	16.67%	0.00%	0.00%	8.33%	66.67%	8.33
Trial 6	0.00%	15.38%	7.69%	7.69%	15.38%	30.77%	23.08
Trial 7	0.00%	30.77%	15.38%	7.69%	15.38%	30.77%	0.00

Table 5. Matrix of accuracies from average clustering performed in Figure 47.

To further see if the data can be grouped appropriately, the channel with the greatest significance (channel 5 in the case of oxy and deoxyhemoglobin) was used to perform the clustering analysis. Similar to the results seen in Figure 47 and Table 5, Figure 48 and Table 6 do not show that any trial is more dominant in any of the clusters.

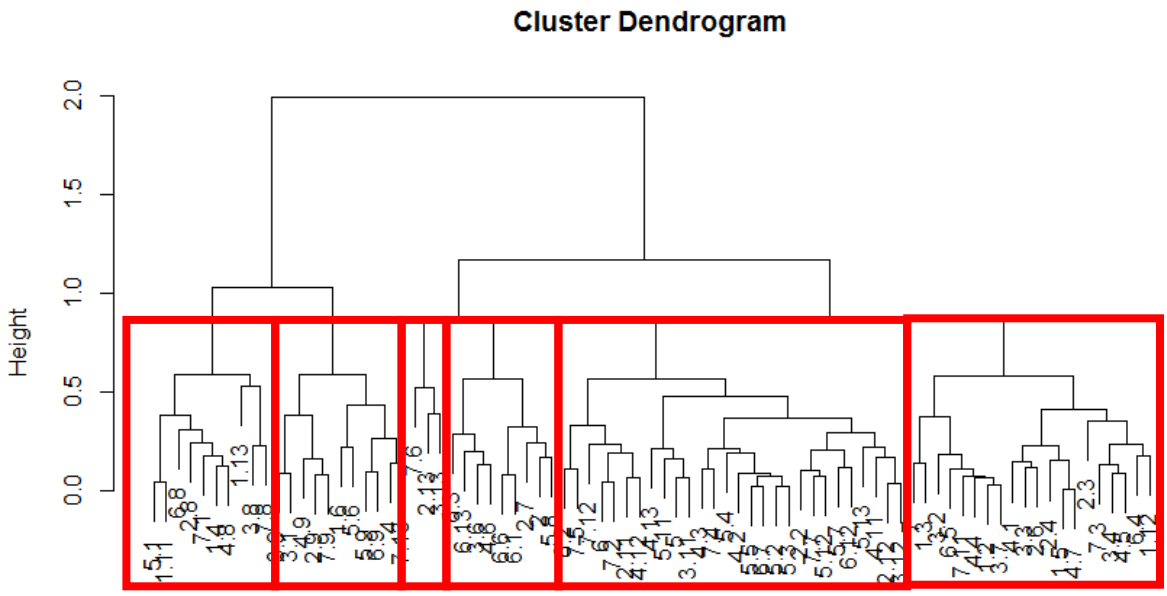


Figure 48. Average clustering analysis using channel 5 from the change in deoxyhemoglobin and channel 5 from the change in oxyhemoglobin; cluster 1 is the leftmost cluster with the clusters following sequentially ending with cluster 5 being the rightmost cluster.

	Cluster 1	Cluster2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Trial 1	42.86%	14.29%	0.00%	0.00%	0.00%	42.86%
Trial 2	9.09%	9.09%	9.09%	18.18%	27.27%	27.27%
Trial 3	9.09%	18.18%	9.09%	9.09%	18.18%	36.36%
Trial 4	7.69%	15.38%	0.00%	7.69%	38.46%	30.77%
Trial 5	7.14%	14.29%	0.00%	7.14%	64.29%	7.14%
Trial 6	8.33%	8.33%	0.00%	33.33%	33.33%	16.67%
Trial 7	15.38%	15.38%	7.69%	0.00%	38.46%	23.08%

Table 6. Matrix of accuracies from average clustering performed in Figure 48.

As this analysis was an exploratory analysis, k-means clustering was also used to see if any information could be obtained by setting the number of clusters that the data should be divided into. Table 7 shows the k-means clustering analysis performed for the fNIRS signals from subjects who underwent the experimental process in AIM 1 for 4°C, with a cutoff of two clusters. Table 7 shows the matrix of accuracies based on trials. The channels of significance for oxyhemoglobin (channels 2, 3, 5 and 6) and deoxyhemoglobin (channels 3, 5 and 6) were used for this analysis. What this shows is how well the clustering algorithm could differentiate between different trials. From the data in Table 7, while trials 1 and 5 seem to have a high percentage of data gathering in cluster 1, there is no apparent pattern that is observed with regards to specific trials (early or late) belonging to specific clusters.

	Cluster 1	Cluster 2
Trial 1	81.82%	18.18%
Trial 2	41.67%	58.33%
Trial 3	69.23%	30.77%
Trial 4	69.23%	30.77%
Trial 5	92.31%	7.69%
Trial 6	58.33%	41.67%
Trial 7	69.23%	30.77%

Table 7. Matrix of accuracies from k-means clustering (cutoff set at 2 clusters) using channels 3, 5 and 6 from the change in deoxyhemoglobin and channels 2, 3, 5 and 6 from the change in oxyhemoglobin.

Table 8 shows the matrix of accuracies based on trials as well using k-means clustering, however the cutoff here was set at 7 trials. The channels of significance for oxyhemoglobin (channels 2, 3, 5 and 6) and deoxyhemoglobin (channels 3, 5 and 6) were used for this analysis. Similar to the data from table 7, there is no apparent pattern that is observed with regards to specific trials (early or late) belonging to specific clusters.

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**	**Cluster 7**
Trial 1	9.09%	9.09%	18.18%	18.18%	27.27%	0.00%	18.18%
Trial 2	0.00%	16.67%	8.33%	16.67%	16.67%	25.00%	16.67%
Trial 3	0.00%	15.38%	15.38%	38.46%	23.08%	0.00%	7.69%
Trial 4	0.00%	7.69%	38.46%	15.38%	23.08%	7.69%	7.69%
Trial 5	0.00%	23.08%	0.00%	15.38%	30.77%	15.38%	15.38%
Trial 6	8.33%	0.00%	16.67%	33.33%	16.67%	0.00%	25.00%
Trial 7	7.69%	0.00%	0.00%	38.46%	30.77%	15.38%	7.69%

Table 8. Matrix of accuracies from k-means clustering (cutoff set at 7 clusters) using channels 3, 5 and 6 from the change in deoxyhemoglobin and channels 2, 3, 5 and 6 from the change in oxyhemoglobin.

Furthering these two analyses, the k-means clustering was repeated, using only the most significant channel in the case of deoxyhemoglobin (channel 5) and oxyhemoglobin (channel 5). Results of this are seen in table 9 and table 10. In both cases, even though majority of cases tend to congregate to cluster 2, there is no apparent pattern that would identify that the data were significantly different enough to be separated through clustering.

	Cluster 1	**Cluster 2**
Trial 1	27.27%	72.73%
Trial 2	41.67%	58.33%
Trial 3	38.46%	61.54%
Trial 4	23.08%	76.92%
Trial 5	23.08%	76.92%
Trial 6	16.67%	83.33%
Trial 7	46.15%	53.85%

Table 9. Matrix of accuracies from k-means clustering (cutoff set at 2 clusters) using channel 5 from the change in deoxyhemoglobin and channel 5 from the change in oxyhemoglobin.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Trial 1	18.18%	9.09%	0.00%	27.27%	9.09%	18.18%	18.18%
Trial 2	16.67%	16.67%	8.33%	25.00%	16.67%	8.33%	8.33%
Trial 3	23.08%	15.38%	0.00%	30.77%	15.38%	15.38%	0.00%
Trial 4	7.69%	7.69%	30.77%	0.00%	15.38%	38.46%	0.00%
Trial 5	15.38%	7.69%	23.08%	15.38%	7.69%	30.77%	0.00%
Trial 6	0.00%	0.00%	41.67%	8.33%	8.33%	33.33%	8.33%
Trial 7	0.00%	23.08%	7.69%	30.77%	15.38%	23.08%	0.00%

Table 10. Matrix of accuracies from k-means clustering (cutoff set at 7 clusters) using channel 5 from the change in deoxyhemoglobin and channel 5 from the change in oxyhemoglobin.

4.4 Discussion

In the brief exploratory analysis conducted in this investigation to better visualize the data, the results from the clustering analysis do not show any definitive differentiation between trials when it comes to grouping the data. This can be interpreted to mean that the hemodynamic response from any specified trial (trial 1, trial 2, trial 3, etc.), was not specific enough to allow the clustering algorithm to train on the data and efficiently separate it. This suggests that the changes after initial exposure to a stimulus are subtle. Identifying two different processes, fast (initial) adaptation, and the subsequent fine tuning that occurs during repeated exposure to bring the body back to stability (Hollins et al., 2011). The rationale for this could include several different things. First, as there were only a small number of subjects (n=15), there may not be enough data for the algorithm to appropriately train on, and thus separate the data. More subjects would help to drive up the significance, or in the case of clustering, dominance of specific trials in specific clusters would be shown. Secondly, there may need to be more preparation of the data prior to clustering. Using functional data analysis before clustering separation may create a better "definition" of what each trial should look like and better inform the clustering (Pourshoghi et al., 2016; Tak & Ye, 2014). Thirdly, adding additional features and using recursive feature extraction will not only focus on significance, but it will have a greater number of features to choose from (Pourshoghi et

al., 2016). Adding this analysis prior to the clustering will give a greater number of features to test from, providing more opportunities for the algorithm find efficient biomarker features for trial separation.

The hypothesis that earlier trials would be separated from later trials failed. This was unexpected given that based on the results from AIM 1, trial 1 impacted the hemodynamic response enough to change the significance of the data when removed from analysis. If this is the case, we at minimum would have expected trial 1 data to be grouped together. With this not being the case, further studies should be conducted with more subjects. This would show if it is simply more data that is needed for clustering to be done appropriately, or if other unsupervised machine-learning techniques should be used for automated data separation. In interpreting these results, the goal to separate the data into groups based on trials was not achieved, but more information about the data, subject number, and quality of the information, can inform future studies to better understand how to separate the data.

4.5 Acknowledgement

We would like to thank Dr. Ahmad Pourshoghi for his work in feature selection and machine learning analysis when analyzing fNIRS signals. This work is integral in the effort towards moving the use of fNIRS in a clinical setting as a diagnostic tool.

Chapter 5

5.1 Summary

Pain is one of the least well understood phenomena in modern medicine (Råheim & Wench, 2006). There are several components to it that make it difficult to understand. In one regard, pain is the result of some external event. From the external event, when the body's defense system goes into action, pain is part of the healing process that allows the body to repair the damage that is done. Pain can also be the result of neuronal degradation or physiological changes in the body, that do not necessarily occur as the result of external happenings. Further, there is the emotional component of pain to also consider (Phillips et al., 2003). Given these differences, it is difficult to find the appropriate tool to use in clinical settings when treating patients with pain. In the last couple of decades, with the advancements in technology as well as data analysis improvements, there has been a paralleled increase in research done in the field of pain; furthermore, as non-invasive modalities become the standard in medicine, imaging modalities have led the field in breakthroughs when it comes to measuring pain. There have been a series of goals that each of these modalities attempt to achieve, and this has led pain research to where it is today.

One of these goals is to find biomarkers (Borsook, 2011) that are coupled with the body's pain response. Biomarkers, like their name suggests, are markers in the biological make up of individuals that are linked with physiological processes. Their importance is in their linkage, because the linkage allows different physiological processes to be followed, monitored, and appropriately treated when something goes wrong. Without these in place, it would make treating bodily ailments more difficult, but taking this approach to investigating pain is one of the first steps in finding physiological features, that are best situated to aide physicians in diagnosing and treating pain.

In the first study conducted in this analysis in AIM 1, the study had the goal of measuring the hemodynamic response to noxious and innocuous stimuli, using the biomarkers of changes in oxy and deoxyhemoglobin. In using multilevel modeling to fit a model that estimates the change over time to repeated exposure, as expected, in the case of 4 degrees Celsius over 7 trials for deoxyhemoglobin, 67% of the channels showed a significant reduction in magnitude over time to the stimulus presented. Upon removing the first trial and conducting the same analysis, it became apparent that the initial exposure greatly influences adaptation and is where the majority of adaptation occurs (only 17% of channels showing significant reduction in response over time). For 4 degrees Celsius result for oxyhemoglobin, 50% of the channels demonstrated a significant reduction in response over time through using multilevel modeling. Overall, informing us that while adaptation does occur when exposed to a stimulus repeatedly, in future works, one may consider focusing on select channels of interest. This continues to further expand upon the studies that aim to identify useful biomarkers for monitoring pain.

The results of AIM 2 showed the utility of these biomarkers in how they compared to the perception of pain. In looking at correlating pain scores to trial number in AIM 2, linear regression showed that while there was a trend in the data that the greater the change in oxy or deoxyhemoglobin, the higher the pain score. This warrants further studies with greater subject numbers to see if significance in the trend of the data can be obtained. While hemodynamics in the prefrontal cortex region may be a robust biomarker for objectively monitoring pain, there is not a direct relationship between perceived adaptation and the objective response – highlighting a difference between the physical pain experience, and the behavioral component encompassing pain perception.

An additional goal for improving the utilities of these modalities in monitoring pain is finding an effective method of using the biomarkers identified in the first goal. While this may seem intuitive, several factors are taken into consideration – combining the data collected using the fNIRS system, with different modes of analyzing this data. This was at the center of the exploratory analysis of AIM 3. The focus here was on clustering the data, and in performing this analysis, no significant results were revealed. The table of matrices show that the hemodynamic change differences between trials was not enough for clustering to separate the data into trial groups. Similar to the results from AIM 2, this suggests that more subjects should be studied with more data to refine the results.

Our measurements based on near infrared spectroscopy (fNIRS) are in support of the idea that objective biomarkers can be obtained from the fNIRS signal. Not only this, but previous studies in our lab, such as initially investigating fNIRS and its ability to monitor the response to pain (Barati et al., 2013), and even further investigating how this data can be useful in a clinical setting through automating the results into useful information (Pourshoghi et al., 2016), in conjunction with previous literature and results shown here, show that repeated exposure to noxious stimuli results in adaptation.

The results discussed throughout this thesis suggest the use of the fNIRS imaging modality to objectively assess the adaptation to stimuli from repeated exposure. Based on the use of fNIRS in the prefrontal cortex region, measurements of the relative change of oxy and deoxyhemoglobin over time were explored to investigate the change over time that occurs as a result of repeated exposure. When presented with a strong enough initial stimulus, we saw that adaptation was readily observable, and continued steadily as repeated trials were employed. Comparing this with

an initial stimulus that is not painful yielded results that showed no adaptation, suggesting that if there is not a strong enough initial stimulus, there is no adaptation to occur.

Based on these findings and in looking at the other measures of perception taken (State Trait Anxiety Inventory and McGill Pain Questionnaire), it suggests that fNIRS may be a promising tool to observe adaptation over time, and that there are clear trends of how the fNIRS signal relates to measures of perception; moreover, it suggests that initial exposure plays a key role in adaptation over time to a stimulus.

5.2 Future Work

While there has been an increased level of interest in fNIRS in regards to the monitoring of pain, there is still a great amount of work to be done. Many of the technologies that provide accurate information for ailment diagnosis such as positron emission tomography (PET), single photon emission computed tomography (SPECT), computerized tomography (CT), and magnetic resonance imaging (MRI) are extremely effective in tracking biomarkers for pain as well as identifying which body parts are impacted by the pain response. Despite their accuracy however, these systems are lacking in their ease of use. Each of the systems identified above are extremely expensive for hospitals or clinics to obtain, and even more, to run tests using these systems costs an exorbitant amount of money. Thus, these shortcomings have given way to other modalities that provide similar information, but are more cost effective and can be used routinely in a clinical setting. Tools such as ultra sound, laser dopplar flowtometry, and near infrared spectroscopy have gained traction in the field of pain research. One of the problems however is that these devices are initially not able to provide the same quality of information, which has caused a growth in improving the data processing of the information provided by these modalities post-use. As a result, the majority of the work until now has focused on the feasibility of using fNIRS to assess

relative pain experienced, the analysis of the data, as well as generating algorithms through machine learning processes to get one step closer to use of fNIRS in a clinical setting. Improvements that can be made upon the work done thus far include:

Phantom limb pain (or similar pain experiences) – When observing phantom limb pain, there is no observable physical component to the pain, but rather a psychological component. Seeing if fNIRS can readily be used as a tool in this regard would prove that it is not limited to physical pain experiences but also psychological pain experiences. This would be an expansion of the work discussed in this thesis as well as other works surrounding the perception of pain.

Multi-distance optode analysis – Many studies with fNIRS that use multi-distance detectors (near versus far), place greater emphasis on the reliability of the far detectors that penetrate to the cortical tissue, versus the near detector which encompasses the signal from the skin response. Not much is known about how the skin response influences the signal. Using techniques such as principal component analysis or independent component analysis will yield a greater understanding of the information received by the far detectors and how to improve signal quality.

5.3 Clinical Applications

The investigation highlighted in the scope of this thesis surrounding the adaptation to repeated exposure to stimuli can have impact in the clinical settings in the following ways:

- When dealing with patients who have mild to chronic pain problems, understanding how they adapt to pain can give information on how to appropriately treat the pain. If patients adapt well to pain, the problem may not lie solely in the pain receptors (for chronic pain patients these receptors often stay activated continuously), but rather physicians may explore other treatment options to yield a more evidence based approach to practicing pain

management medicine. (Hirsch, Charpie, Ohye, & Gurney, 2010; Obrig, 2014; Smith, 2011)

- This also suggests how fNIRS can be used as a diagnostic tool in that the scope of this research included healthy individuals who react appropriately to pain. If in the clinic, an otherwise healthy person undergoes this same test and does not adapt to pain after exposure to noxious stimuli as was seen throughout the results in this thesis, this could be an indicator in that their body does not react to pain in a healthy manner. Why this is important is that pain is a phenomenon that our body needs to let us know when we are in danger or experiencing an unusual change to our system.
- With the size, mobility and robustness of the fNIRS system, it can readily be used in a clinical setting with fast and reliable results, which is why the research here, as well as continuing research in applying fNIRS to pain brings us closer and closer to having fNIRS as an aide in the diagnosis and treatment of pain.

References

1. American Cancer Society. (2014). Cancer treatment & survivorship facts & figures 2014-2015.

2. Bandura, a, & Wood, R. (1989). Effect of perceived controllability and performance standards on self-regulation of complex decision making. *Journal of Personality and Social Psychology*, *56*(5), 805–814. https://doi.org/10.1037/0022-3514.56.5.805

3. Barati, Z., Shewokis, P. A., Izzetoglu, M., Polikar, R., Mychaskiw, G., & Pourrezaei, K. (2013). Hemodynamic response to repeated noxious cold pressor tests measured by functional near infrared spectroscopy on forehead. *Ann Biomed Eng*, *41*(2), 223–237. https://doi.org/10.1007/s10439-012-0642-0

4. Boas, D. a, Elwell, C. E., Ferrari, M., & Taga, G. (2014). Twenty years of functional near-infrared spectroscopy: introduction for the special issue. *NeuroImage*, *85 Pt 1*, 1–5. https://doi.org/10.1016/j.neuroimage.2013.11.033

5. Bodnar, R. J., Kelly, D. D., Spiaggia, A., & Glusman, M. (2016). Stress-induced analgesia : Adaptation following chronic cold water swims Stress-induced analgesia : Adaptation following chronic cold water swims, (February). https://doi.org/10.3758/BF03336847

6. Borsook, D., Sava, S., & Becerra, L. (2010). The pain imaging revolution: advancing pain into the 21st century. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *16*(2), 171–185. https://doi.org/10.1177/1073858409349902

7. Brock, G., Pihur, V., Datta, S., & Datta, S. (2008). {{\tt clValid}}: An {R} Package for Cluster Validation. *Journal of Statistical Software*, *25*(4), 1–22. Retrieved from http://www.jstatsoft.org/v25/i04

8. Brodersen, K. H., Wiech, K., Lomakina, E. I., Lin, C. S., Buhmann, J. M., Bingel, U., … Tracey, I. (2012). Decoding the perception of pain from fMRI using multivariate pattern analysis. *NeuroImage*, *63*(3), 1162–1170. https://doi.org/10.1016/j.neuroimage.2012.08.035

9. Brown, J. E., Chatterjee, N., Younger, J., & Mackey, S. (2011). Towards a physiology-based measure of pain: Patterns of human brain activity distinguish painful from non-painful thermal stimulation. *PLoS ONE*, *6*(9). https://doi.org/10.1371/journal.pone.0024124

10. Casey, K. L., Minoshima, S., Morrow, T. J., & Koeppe, R. a. (1996). Comparison of human cerebral activation pattern during cutaneous warmth, heat pain, and deep cold pain. *Journal of Neurophysiology*, *76*(1), 571–581.

11. Chambers, R. a, Bremner, J. D., Moghaddam, B., Southwick, S. M., Charney, D. S., & Krystal, J. H. (1999). Glutamate and post-traumatic stress disorder: toward a psychobiology of dissociation. *Seminars in Clinical Neuropsychiatry*, *4*(4), 274–281. https://doi.org/10.153/SCNP00400274

12. Chance, B., Anday, E., Nioka, S., Zhou, S., Hong, L., Li, C., … Thomas, R. (1998). A novel method for fast imaging of brain function , non-invasively , with light, *2*(10), 4324–4332.

13. Chizh, B. a, & Hobson, A. R. (2007). Using objective markers and imaging in the development of novel treatments of chronic pain. *Expert Review of Neurotherapeutics*, *7*(5), 443–447. https://doi.org/10.1586/14737175.7.5.443

14. Denk, F., McMahon, S. B., & Tracey, I. (2014). Pain vulnerability: a neurobiological perspective. *Nature Neuroscience*, *17*(2).

15. Diatchenko, L., Nackley, A. G., Tchivileva, I. E., Shabalina, S. a., & Maixner, W. (2007). Genetic architecture of human pain perception. *Trends in Genetics*, *23*(12), 605–613. https://doi.org/10.1016/j.tig.2007.09.004

16. Dickenson, A. (2011). The neurobiology of chronic pain states. *Anaesthesia and Intensive Care Medicine*, *14*(11), 484–487. https://doi.org/10.1016/j.mpaic.2013.08.008

17. Disbrow, E., Buonocore, M., Antognini, J., Carstens, E., & Rowley, H. a. (1998). Somatosensory cortex: A comparison of the response to noxious thermal, mechanical, and electrical stimuli using functional magnetic resonance imaging. *Human Brain Mapping*, *6*(3), 150–159. https://doi.org/10.1002/(SICI)1097-0193(1998)6:3<150::AID-HBM4>3.0.CO;2-2

18. Donald, D. (2000). Psychological and neural mechanisms of the affective dimension of pain.

19. Goldstein N., R. . V. (2002). Drug Addiction and Its Underlying Neurobiological Basis - Neuroimaging Evidence for the Involvement of the Frontal Cortex. *Psychiatry*, *159*(10), 21.

20. Hart, P. D. (2003). Americans Talk About Pain: A Survey Among Adults Nationwide Conducted for Research!America.

21. Hirsch, J. C., Charpie, J. R., Ohye, R. G., & Gurney, J. G. (2010). Near infrared spectroscopy (NIRS) should not be standard of care for postoperative management. *Seminars in Thoracic and Cardiovascular Surgery. Pediatric Cardiac Surgery Annual*, *13*(1), 51–4. https://doi.org/10.1053/j.pcsu.2010.01.005

22. Hodges, P. W., & Tucker, K. (2011). Moving differently in pain: A new theory to explain

the adaptation to pain. *Pain*, *152*(SUPPL.3), S90–S98. https://doi.org/10.1016/j.pain.2010.10.020

23. Hollins, M., Harper, D., & Maixner, W. (2011). Changes in pain from a repetitive thermal stimulus : The roles of adaptation and sensitization. *Pain*, *152*(7), 1583–1590. https://doi.org/10.1016/j.pain.2011.02.049

24. Institute of Medicine of the National Academies. (2011). Relieving Pain in America - A Blueprint for Transforming Prevention, Care, Education, and Research. *Pain Research 2011 Report Brief*, 4. Retrieved from www.iom.edu/relievingpain

25. Kabat-Zinn, J. (1982). An outpatient program in behavioral medicine for chronic pain patients based on the practice of mindfulness meditation: theoretical considerations and preliminary results. *General Hospital Psychiatry*, *4*(1), 33–47. https://doi.org/10.1016/0163-8343(82)90026-3

26. Keogh, E., & Herdenfeldt, M. (2002). Gender , coping and the perception of pain, *97*, 195–201.

27. Kwok, O.-M., Underhill, A. T., Berry, J. W., Luo, W., Elliott, T. R., & Yoon, M. (2008). Analysing longitudinal data with multilevel models: An example with individuals living with lower extremity intra-articular fractures. *Rehabilitation Psychology*, *53*(3), 370–386. https://doi.org/10.1037/a0012765.Analyzing

28. Ledoux, J. E. (2000). Emotion circuits in the brain. *New York*, 155–184.

29. Loeser, J. D., & Melzack, R. (1999). Pain : an overview, *353*, 1607–1609.

30. Marquand, A., Howard, M., Brammer, M., Chu, C., Coen, S., & Mourão-Miranda, J. (2010). Quantitative prediction of subjective pain intensity from whole-brain fMRI data using Gaussian processes. *NeuroImage*, *49*(3), 2178–2189. https://doi.org/10.1016/j.neuroimage.2009.10.072

31. Melzack, R. (1987). The short-form McGill Pain Questionnaire. *Pain*, *30*, 7.

32. Melzack, R. (2001). Pain and the neuromatrix in the brain. *Journal of Dental Education*, *65*(12), 1378–1382.

33. Melzack, R., & Wall, P. D. (1996). Pain mechanisms: A new theory. *Pain Forum*, *5*(1), 3–11. https://doi.org/10.1016/S1082-3174(96)80062-6

34. Nair, A., & Bonneau, R. H. (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *Journal of Neuroimmunology*, *171*(1–2), 72–85. https://doi.org/10.1016/j.jneuroim.2005.09.012

35. Obrig, H. (2014). NIRS in clinical neurology - a “promising” tool? *NeuroImage*, *85 Pt 1*, 535–46. https://doi.org/10.1016/j.neuroimage.2013.03.045

36. Ohsawa, H., Okada, K., Nishijo, K., & Sato, Y. (1995). Neural mechanism of depressor responses of arterial pressure elicited by acupuncture-like stimulation to a hindlimb in anesthetized rats. *Journal of the Autonomic Nervous System*, *51*(1), 27–35. https://doi.org/10.1016/0165-1838(95)80004-T

37. Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003). Neurobiology of Emotion Perception I : The Neural Basis of Normal Emotion Perception. https://doi.org/10.1016/S0006-3223(03)00168-9

38. Porro, C. a, Cettolo, V., Francescato, M. P., & Baraldi, P. (1998). Temporal and intensity coding of pain in human cortex. *Journal of Neurophysiology*, *80*(6), 3312–3320.

39. Pourshoghi, A., Zakeri, I., & Pourrezaei, K. (2016). Application of functional data analysis in classification and clustering of functional near-infrared spectroscopy signal in response to noxious stimuli. *Journal of Biomedical Optics*, *21*(10), 101411. https://doi.org/10.1117/1.JBO.21.10.101411

40. Råheim, M., & Wench, H. (2006). Lived Experience of Chronic Pain and Fibromyalgia : Women ’ s Stories From Daily Life, *16*(6), 741–761. https://doi.org/10.1177/1049732306288521

41. Rainville, P. (2002). Brain mechanisms of pain affect and pain modulation. *Current Opinion in Neurobiology*, *12*(2), 195–204. https://doi.org/10.1016/S0959-4388(02)00313-6

42. Ranger, M., & Gélinas, C. (2014). Innovating in Pain Assessment of the Critically Ill: Exploring Cerebral Near-Infrared Spectroscopy as a Bedside Approach. *Pain Management Nursing*, *15*(2), 519–529. https://doi.org/10.1016/j.pmn.2012.03.005

43. Ravn, P., Frederiksen, R., Skovsen, A. P., Christrup, L. L., & Werner, M. U. (2012). Prediction of pain sensitivity in healthy volunteers. *J Pain Res*, *5*, 313–326. https://doi.org/10.2147/JPR.S33925

44. Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Adams, R. J., Berry, J. D., Brown, T. M., … Wylie-Rosett, J. (2011). Heart disease and stroke statistics-2011 update: A report from the American Heart Association. *Circulation*, *123*(4), 18–209. https://doi.org/10.1161/CIR.0b013e3182009701

45. Romero, L. M., Dickens, M. J., & Cyr, N. E. (2009). Hormones and Behavior The reactive scope model — A new model integrating homeostasis , allostasis , and stress. *Hormones and Behavior*, *55*(3), 375–389. https://doi.org/10.1016/j.yhbeh.2008.12.009

46. Salomons, T., & Johnstone, T. (2007). Individual differences in the effects of perceived controllability on pain perception: critical role of the prefrontal cortex. *Journal of Cognitive …*, 993–1003. https://doi.org/10.1162/jocn.2007.19.6.993

47. Smith, M. (2011). Shedding light on the adult brain: a review of the clinical applications of near-infrared spectroscopy. *Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences*, *369*(1955), 4452–69. https://doi.org/10.1098/rsta.2011.0242

48. Soetanto J.; Wong, T., A. . C. (2006). Are There Gender Differences in Pain Perception? *Neuroscience Nursing*, *38*(3), 5.

49. Spielberger R.; Lushere, R., C. . G. (1971). State-Trait Anxiety Inventory. *Professional Psychology*, 2.

50. Tak, S., & Ye, J. C. (2014). Statistical analysis of fNIRS data: a comprehensive review. *NeuroImage*, *85 Pt 1*, 72–91. https://doi.org/10.1016/j.neuroimage.2013.06.016

51. Taylor, J. A., & Taylor, J. A. (1956). LEVEL OF CONDITIONING AND INTENSITY OF THE ADAPTATION STIMULUS. *Journal of Experimental Psychology*, *51*(2).

52. Tracey, I., Becerra, L., Chang, I., Breiter, H., Jenkins, L., Borsook, D., & González, R. G. (2000). Noxious hot and cold stimulation produce common patterns of brain activation in humans: A functional magnetic resonance imaging study. *Neuroscience Letters*, *288*(2), 159–162. https://doi.org/10.1016/S0304-3940(00)01224-6

53. Villringer, A., & Chance, B. (1997). Non-invasive optical spectroscopy and imaging of human brain function. *Trends in Neurosciences*, *20*(10), 435–442.

54. Wager, T. D., Atlas, L. Y., Leotti, L. a, & Rilling, J. K. (2011). Predicting individual differences in placebo analgesia: contributions of brain activity during anticipation and pain experience. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(2), 439–452. https://doi.org/10.1523/JNEUROSCI.3420-10.2011

55. Wager, T. D., Atlas, L. Y., Lindquist, M. a, Roy, M., Woo, C.-W., & Kross, E. (2013). An fMRI-based neurologic signature of physical pain. *The New England Journal of Medicine*, *368*(15), 1388–97. https://doi.org/10.1056/NEJMoa1204471

56. Yang, W., Dall, T. M., Halder, P., Gallo, P., Kowal, S. L., Hogan, P. F., & Petersen, M. (2013). Economic costs of diabetes in the U.S. in 2012. *Diabetes Care*, *36*(4), 1033–1046. https://doi.org/10.2337/dc12-2625

VITA
DARYL OMIRE-MAYOR

EDUCATION:

Ph.D in Biomedical Science, June 2017
Master of Science in Biomedical Science, March 2014
Drexel University

Bachelor of Science in Biological Sciences, December 2011
Bachelor of Arts in Economics, December 2011
Meyerhoff Scholar
University of Maryland, Baltimore County (UMBC)

SKILLS:

- **Lab Skills:** PCR, HPLC, Gel Electrophoresis, PeakNet® (IC System), Microdialysis, High Performance Liquid Chromatography (HPLC), Pulsed Electrochemical Detection (PED), Gas Chromatography, Functional Near-Infrared Spectroscopy (fNIRS), Electrocardiography (ECG), Electroencephalogram (EEG), Laser Speckle Imaging (LSI)
- **Computer:** Microsoft Office (Power Point, Excel, Word), Intermediate Programming in R, Intermediate Programming in Matlab
- **Languages:** Intermediate Spanish, Conversational Mandarin

HONORS AND AWARDS:

- Meyerhoff Scholar (2007 – 2011)
- UMBC Undergraduate Analytical Chemist of the Year (Spring 2009)
- Distinguished Service Award – Philadelphia Louis Stokes Alliance for Minority Participation (April 2015)
- Teaching Assistant Excellence Award (2016 Winner)
- NSF Fellowship to attend the IEEE EMBS 15th International Summer School on Biocomplexity, Biodesign & Bioinnova: from Gene to System (June 2016)

RESEARCH EXPERIENCE:

Research Assistant, Drexel University Biomedical Engineering Department
Advisor: Kambiz Pourrezaei, PhD
Philadelphia, PA. 01/12 – Present.

- Use Functional Near-Infrared imaging as a spectroscopic modality for neuro-imaging that allows for a continuous and non-invasive monitoring of changes in blood oxygenation that correlate to human brain function.
- Study pain tolerance and pain threshold through optical brain imaging while performing tests such as the cold pressor test.

Research Assistant, Shanghai Jiao Tong University Biomedical Engineering Department
PI: Shanbao Tong, PhD
Shanghai, China. 10/14 – 03/15.
A short-term research experience (6 months) where I joined the research team in the Neural Engineering Laboratory at the Med-X Research Institute of Shanghai Jiao Tong University, School of Biomedical Engineering.

- Study cortical plasticity in amputees' both pre and post amputation using Laser Speckle Imaging technology.
- Plasticity changes over time using FES with different stimulation parameters coupled with LSI.
- Find correlations between the rat model of amputation and plasticity and suggest a possibly mechanism for change in plasticity for humans.

Lab Technician, Walter Reed Army Institute of Research
PI: Kacey Morasch, PhD
Silver Spring, MD. 02/11 – 02/12.

- Use electroencephalogram (EEG) recordings to monitor sleep of subjects, and score sleep to interpret quality of sleep obtained by subjects.
- Application and maintenance of polysomnographic (PSG) electrodes and equipment, and electrocardiogram (EKG) electrodes and equipment.
- Preparation of biological samples for processing (blood and urine).
- Screening potential participants for research studies.

Undergraduate Research Assistant, UMBC Chemistry Department
PI: William R. LaCourse, PhD
Baltimore, MD. 02/08 – 02/11.

- Conduct research project that dealt with quantifying biomarkers in physiological fluids. Assess sugars' recoveries following Microdialysis – High Performance Anion Exchange Chromatography – Pulsed Electrochemical Detection to show whether size and/or charge make any difference in a sugars' recovery.
- Keep accurate documentation, which leads to better analytical methods for carbohydrate-based biomarkers and a deeper understanding of microdialysis separation.

Research Intern, NIH/NIDDK Short Term Education Program for Underrepresented Persons Program, Baltimore, MD. Summer 2008.
A summer research-training program at the University of Maryland Baltimore County that was developed for talented undergraduates who are dedicated to the advancement of underrepresented groups in the sciences and mathematics.

- Obtained state-of-the-art scientific research experience to better understand biomedical research careers in areas of specific interest to the NIDDK.
- In the Department of Chemistry at UMBC, I completed a project focused on diabetes in where I investigated the process of sugar recovery.

PUBLICATIONS:

- Zhao, L., Li, Y., Li, H., **Omire-Mayor, D.**, Tong, S. "The cerebral blood flow response dependency on stimulus pulse width is affected by stimulus current amplitude-a study of activation flow coupling." *Engineering in Medicine and Biology Society (EMBC), 2015 37th Annual International Conference of the IEEE*. IEEE, 2015.
- Li, Hangdao, et al. "Low-intensity (400 mW/cm 2, 500 kHz) pulsed transcranial ultrasound preconditioning may mitigate focal cerebral ischemia in rats." *Brain Stimulation* (2017).

SCIENTIFIC WORKSHOPS ATTENDED:

IEEE EMBS 15th International Summer School on Biocomplexity, Biodesign &Bioinnova
Seferihisar, Izmir, Turkey. June 24-30, 2016

- The summer school exposes undergraduate and graduate biological science and bioengineering students to new approaches of the mathematical and computational challenges in Systems Biology and the new directions in computational biology, bioinformatics and molecular engineering research and to facilitate rapid diffusion of these mathematical and computational tools in the biological sciences.

IEEE EMBS 11th Annual International Summer School on Biomedical Imaging.
Brittany, France. June 16-24 2014

- The summer school is aimed at offering to the best young researchers (PhD students, Post-doctorate, Engineers from industry, Physicians) involved in biomedical imaging an exceptional opportunity to meet, discuss and learn from leading lecturers in an informal atmosphere.
- Research presented: **Omire-Mayor, D.**, Pourshoghi, A., Lee, K., Aguinaldo, A., Pourrezaei, K. The Effect of Physiological Changes on Pain Perception and Detection using Non-Invasive fNIRS. IEEE EMBS 11th Annual International Summer School on Biomedical Imaging. Brittany, France. June 2014.

IEEE EMBS International Summer School on Neural Engineering.
Shanghai, China. July 7-14, 2013

- This summer school invites leading scientists and experts in neural engineering to give lectures and seminar talks, as well as organize site visits to clinical facilities. The two subtopics of the summer school include: Neuroimaging and Brain & Neural Interface
- Research presented: **Omire-Mayor, D.**, Lee, K., Mulay, P., Ramesh, M., Barati, Z., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measured by fNIR. IEEE EMBS International Summer School on Neural Engineering. Shanghai, China. July 2013.

SCIENTIFIC CONFERENCES ATTENDED:

- **Omire-Mayor, D.**, Pourshoghi, A., Wong, A., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measured by Functional Near Infrared Spectroscopy. Annual Biomedical Research Conference for Minority Students. Seattle, WA. November 2015.
- **Omire-Mayor, D.**, Pourshoghi, A., Wong, A., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measured by Functional Near Infrared Spectroscopy. Society for Neuroscience. Chicago, IL. October 2015. (Invited Oral Presentation)
- **Omire-Mayor, D.**, Pourshoghi, A., Lee, K., Aguinaldo, A., Pourrezaei, K. Assessing the Effect of Physiological Changes on Pain Detection and Perception using Non-Invasive Functional Near Infrared Spectroscopy. Shanghai Jiao Tong University Biomedical Engineering Research Day. Shanghai, China. December 2014.*
- **Omire-Mayor, D.**, Pourshoghi, A., Lee, K., Aguinaldo, A., Pourrezaei, K. The Effect of Physiological Changes on Pain Perception and Detection using Non-Invasive fNIRS. Drexel Research Day. Philadelphia, PA. April 2014.
- **Omire-Mayor, D.**, Pourshoghi, A., Lee, K., Aguinaldo, A., Pourrezaei, K. Assessing the Effect of Physiological Changes on Pain Detection and Perception using Non-Invasive Functional Near Infrared Spectroscopy. University of Pennsylvania Symposium on Challenges & Opportunities in Pain. Philadelphia, PA. December 2013.

- **Omire-Mayor, D.**, Allard, R., Oladosu, B., Morasch, K. The Effect of Acetylcholinesterase Inhibitors on Subjective Sleepiness Scores. Walter Reed Army Institute of Research College Qualified Leaders Symposium. Silver Spring, MD. August 2013.
- **Omire-Mayor D.**, Lee, K., Mulay, P., Ramesh, M., Barati, Z., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measured by fNIR. Drexel Research Day 2013. Philadelphia, PA. April 2013.
- Lee, K., **Omire-Mayor, D.**, Mulay, P., Ramesh, M., Barati, Z., Pourrezaei, K The Effect of Experimental Pain on Hemodynamic Responses Measured by fNIR. Colonial Academic Alliance Undergraduate Research Conference. Newark, DE. April 2013.
- Vyas, R. **Omire-Mayor, D.**, Barati, Z., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measures by fNIRS. Biomedical Engineering Society Fall 2012 Conference. Atlanta, GA. October 2012. (Invited Oral Presentation)
- **Omire-Mayor, D.**, Logan, S., Rupp, T. The Psychomotor Vigilance Test: Reliability and New Administration Formats. Walter Reed Army Institute of Research College Qualified Leaders Symposium. Silver Spring, MD. August 2012.
- **Omire-Mayor, D.**, Vyas R., Sawan, H., Vaghani, M., Lim, E., Barati, Z., Pourrezaei, K. The Effect of Experimental Pain on Hemodynamic Responses Measured by fNIR. University of Pennsylvania Optical Imaging Research Symposium. Philadelphia, PA. June 2012.
- **Omire-Mayor D.**, Logan, S., Rupp, T. The Effect of Sleep Deprivation on Psychomotor Vigilance Performance Based on Social Exposure. Walter Reed Army Institute of Research College Qualified Leaders Symposium. Silver Spring, MD. August 2011.
- **Omire-Mayor, D.**, Wassink, S., LaCourse, W. The Effect of Molecular Characteristics on Sugar Recovery. American Chemical Society (ACS) Fall 2010 Conference. Boston, MA. August 2010.
- **Omire-Mayor, D.**, Wassink, S., LaCourse, W. The Effect of Molecular Characteristics on Sugar Recovery. UMBC Undergraduate Research and Creative Achievement Day. Baltimore, MD. April 2010.
- **Omire-Mayor, D.**, Wassink, S., LaCourse, W. The Effect of Molecular Characteristics on Sugar Recovery. 11th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences. Baltimore, MD. August 2008.
- **Omire-Mayor, D.**, Wassink, S., LaCourse, W. The Effect of Molecular Characteristics on Sugar Recovery. Short-Term Education Program for Underrepresented Persons (STEP-UP) Undergraduate Research Training Symposium. Washington, DC. August 2008.

**Award Received for Excellence in Presentation*

TEACHING EXPERIENCE*:

Teaching Assistant, Drexel Biomedical Engineering Department
Philadelphia, PA. Spring 2014 – Summer 2016.

- Work with professor of Biomedical Engineering 338/538: Biomedical Ethics and Law and assist with grading, course logistics, creation of student assignments and acting as liaison between the course professor and students. (Summer 2016)
- Work with professor of Biomedical Engineering 488/588: Medical Device Development and assist with grading, course logistics as well as structuring of student assignments throughout the course. (Spring 2016)

- Work with professor of Biomedical Engineering 340/825: Health Care and Hospital Administration and assisted with the logistics of weekly presentations by students, as well as keeping all records of grades through the course. (Winter 2016)
- Worked with professor of Biomedical Engineering 510: Biomedical Statistics (Graduate version), and assisted by handling all course logistics and grading. Acted as a resource outside of class for any help that students may have needed throughout the quarter. (Fall 2015)
- Worked with professor of Biomedical Engineering 310: Biomedical Statistics and assisted by serving as a liaison between the professor and students as needed as well as ensuring that all grading for students was complete and done in a timely manner. (Summer 2015)
- Worked with the professor of Biomedical Engineering 588: Medical Device Development and assisted by serving as an administrative assistant for the course including copying, grading exams and ensuring students grades were organized. (Spring 2014)

Teaching Assistant, Drexel Biology Department
Philadelphia, PA. Winter 2014 – Spring 2015.

- Work with the professors of Biology 126: Physiology and Ecology, and assist by facilitating/leading two laboratory sections. (Spring 2015)
- Work with the professors of Biology 126: Physiology and Ecology, and assist by facilitating/leading two laboratory sections. (Spring 2014)
- Worked with the professors of Biology 124: Evolution and Organismal Diversity, and assisted by facilitating/leading two laboratory sections. (Winter 2014)

Enon Coulter Community Development Corporation, Camp Adonai STEM Component
Supervisor: Michelle Rogers, PhD
Philadelphia, PA. Summer 2014.

- Directed the teaching of the science component of this summer camp. The focus was on educating children (7-12 years of age) in the scientific method through the use of engaging activities centered on engineering.

Teaching Assistant, Drexel College of Information Science & Technology
Philadelphia, PA. Fall 2013 – Spring 2014.

- Work with the professors of Information Science 204: Nursing Informatics, and assist by facilitating/leading three laboratory sections.

Teaching Assistant, Drexel Physics Department
Philadelphia, PA. Spring 2012 – Spring 2015.

- Worked with the professor of Physics 101: Fundamentals of Physics I, and assisted by facilitating/leading three discussion sections. (Spring 2015)
- Worked with the professor of Physics 201: Fundamentals of Physics III, and assisted by facilitating/leading two laboratory sections and served as administrative assistant for the course. (Winter 2014)
- Worked with the professor of Physics 201: Fundamentals of Physics III, and assisted by facilitating/leading three laboratory sections. (Fall 2013)
- Worked with the professor of Physics 101: Fundamentals of Physics I, and assisted by facilitating/leading three discussion sections. (Spring 2013)
- Worked with the professor of Physics 154: Introductory Physics III, and assisted by facilitating/leading two discussion sections and one laboratory section. (Fall 2012)

- Worked with the professor of Physics 154: Introductory Physics III, and assisted by facilitating/leading two discussion sections and one laboratory section. (Spring 2012)

Undergraduate Teaching Assistant, UMBC Biology Department
Baltimore, MD. Fall 2009/2010.

- Worked with the professor of the Biology 100 Lab class and assisted wherever help was needed to ensure that the lab section I was assigned to ran smoothly.

VOLUNTEER EXPERIENCES:

Volunteer, West Catholic Preparatory High School
Philadelphia, PA. 12/15 – Present.

- Work with students at all levels: Freshmen, Sophomores and Juniors by tutoring them in Algebra I, Geometry and Algebra II to complement what they learn in the classroom.

Clinic Volunteer, Hahnemann Hospital – Heart Failure, Heart Transplant (Cardiology)
Supervisor: Shelley Hankins, MD
Philadelphia, PA. 04/15 – Present.

- Work under the supervision of Dr. Shelley Hankins where I was exposed to all facets of the Cardiology clinic at Hahnemann hospital, but assisted primarily in the assessment of patients' readiness to participate in clinical trials.

Volunteer, University of Maryland Medical Center Shock Trauma Center
Baltimore, MD. 06/08 – 06/10.

- Worked with doctors and nurses in the Shock Trauma Center of the hospital.
- Performed tasks such as transporting patients, picking up orders from the blood bank, assisting with small procedures such as the putting on of casts, restocking of patient areas and fulfilling patients' needs.

Project HEALTH Volunteer, St. Agnes Hospital
Baltimore, MD. 01/08 – 12/08.

- Program for UMBC service-learning students to **implement public** health **interventions in partnership with St. Agnes Hospital, our university, and community organizations.**
- Acted as a social work intern by helping children and families address the broad spectrum of issues that determine health and well-being.

Shadowing Doctor, Providence Hospital – OB/GYN Department
Supervisor: Tollie Elliot, MD
Washington, D.C. 12/07 – 01/08.

- Worked under the direction of Dr. Tollie Elliot where I was provided clinical exposure to the obstetrician and gynecology primary care clinic as well as labor and delivery.
- It allowed me to see a multitude of procedures both in and out of the OR that relate to patent wellbeing.